Estimating kinetic parameters for single channels with simulation

A general method that resolves the missed event problem and accounts for noise

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ABSTRACT Analysis of currents recorded from single channels is complicated by the limited time resolution (filtering) of the data which can prevent the detection of brief intervals. Although a number of approaches have been used to correct for the undetected intervals (missed events) when identifying kinetic models and estimating parameters, none of them provide a general method which takes into account the true effects of noise and limited time resolution. This paper presents such a method. The approach is to use simulated single-channel currents to incorporate the true effects of filtering and noise on missed events and interval durations. The simulated currents are then analyzed in a manner identical to that used to analyze the experimental currents. An iterative search process using likelihood comparison of two-dimensional dwell-time distributions obtained from the simulated and experimental single-channel currents then allows the most likely rate constants to be determined. The large errors and false solutions that can result from the more typically applied assumptions of no noise and an absolute dead time (idealized filtering) are excluded by the iterative simulation method, and the correlation information contained in the two-dimensional distributions should increase the ability to distinguish among different gating mechanisms. The iterative simulation method is generally applicable to channels which typically open to a single conductance level. For these channels the method places no restrictions on the proposed gating mechanism or the form of the predicted dwell-time distributions.

INTRODUCTION

Analysis of currents recorded from single ion channels provides a powerful means to investigate the mechanism by which channels gate their pores (Colquhoun and Hawkes, 1981; Horn and Lange, 1983). The interpretation of the results can be compromised, however, by both the limited time resolution (filtering) of single-channel currents and by the noise in the current record. Limited time resolution can prevent the shorter duration open-and-shut intervals from being detected. Such missed events can lead to an erroneous increase in the duration of the observed open and shut interval durations (Sachs et al., 1982; Colquhoun and Sigworth, 1983; Wilson and Brown, 1985; Roux and Sauve, 1985; Blatz and Magleby, 1986*a*). Noise can either facilitate or depress the detection of each individual event, depending on the direction of the noise, and these effects of noise do not cancel out (McManus et al., 1987). In addition, large noise peaks can be detected as false events (Colquhoun and Sigworth, 1983).

A number of different methods have been developed to reduce errors introduced by filtering and noise. Empirical approaches can be of some use, (McManus et al., 1987), but lack general applicability. Comparisons of amplitude histograms of currents from simulated and experimental data can be used to account for filtering and noise if the durations of the open and shut intervals are sufficiently brief to yield flickery data (Yellen, 1984; Pietrobon et al., 1989). Such current amplitude methods have proven useful for assumed two-state blocking models (Yellen, 1984; Pietrobon et al., 1989), but lack resolution for more complicated gating mechanisms or for data in which the intervals are not heavily attenuated. The empirical and current amplitude methods also do not directly take into account the time relationships among successive intervals. Such correlation information is crucial to distinguish among models (Horn and Lange, 1983; Jackson et al., 1983; Fredkin et al., 1985; Colquhoun and Hawkes, 1987; Blatz and Magleby, 1989; Ball et al., 1988; Ball and Sansom, 1989).

Most analysis methods derive their kinetic information by comparing observed interval durations with those predicted by matrix methods (Colquhoun and Hawkes, 1981; Horn and Lange, 1983; Fredkin et al., 1985; Roux and Sauve, 1985; Blatz and Magleby, 1986*a*; Ball and Sansom, 1989). Whereas matrix methods have provided powerful tools for advances in our understanding of channel gating (Horn and Vandenberg, 1984; Colquhoun and Sakmann, 1985; Labarca et al., 1985; Blatz and Magleby, 1986*b*), the findings can be limited, as the matrix methods disregard the effects of noise and make unrealistic (idealized) assumptions about the effects of filtering. For example, it is typically assumed that all events less than a certain duration, the dead time, go

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undetected. Such an assumption can lead to false solutions (Colquhoun and Sigworth, 1983; Blatz and Magleby, 1986*b*; Yeo et al., 1988), and is inconsistent with the true effects of filtering, where subthreshold events can be added and detected (Roux and Sauve, 1985). Furthermore, even with simplifying assumptions about filtering, it is often impractical to solve for the desired correlation information with matrix methods.

What is needed is a method to study channel gating mechanism which uses the essential kinetic information in the single-channel current record while taking into account the true effects of filtering and noise on missed events and interval durations. Ideally, this should be a general method which places no restrictions on gating mechanism or the predicted forms of the dwell-time distributions. This paper presents such a method for channels which typically open to a single conductance level. A preliminary report of some of these results has appeared (Magleby and Weiss, 1990*a*).

METHODS

Calculations were performed on a DEC 11/73 computer (Digital Equipment Corp., Marlboro, MA) containing an XP-11 accelerator board (Cheshire Engineering Corp., Pasadena, CA) using FORTRAN 77. The XP-11 board ran about five to six times faster than the DEC 11/73.

Simulating single-channel current records with true filtering and noise

Filtered and noisy single-channel current records were generated as follows (McManus et al., 1987). In the first step, the open-and-shut interval durations that would be observed if there were no noise and unlimited time resolution (no filtering) were generated for a given kinetic scheme and rate constants using methods similar to those in Clay and DeFelice (1983) and Blatz and Magleby (1986*b*). An example of such an idealized single-channel current is shown in Fig. 1 *A*, where upward current steps indicate channel opening.

The idealized single-channel current is then filtered using the method described by Colquhoun and Sigworth (1983) for time-course fitting of single-channel data. The filter response to a positive current step at the time of each channel opening and the filter response to a negative current step at the time of each channel closing are added into an array to give the filtered response. Fig. 1 *B* plots the individual filter responses to positive (upward going) and negative (downward going) current steps, and Fig. 1 *C* plots their sum. Fig. 1 *C* is thus the current record that would be observed after filtering the ideal current record in Fig. 1 *A*. (Details for generating the filter responses are in the following section.)

After obtaining the filtered current record, the next step towards simulating a filtered and noisy single-channel current record is to add noise. Noise in the absence of channel openings is preferably obtained (see below) from the experimental current record to be analyzed. An example of such noise is shown in Fig. 1 *D*; note that the noise record is already filtered. The filtered noise in Fig. 1 *D* is then summed with the filtered current record in Fig. 1 *C* to obtain the filtered and noisy

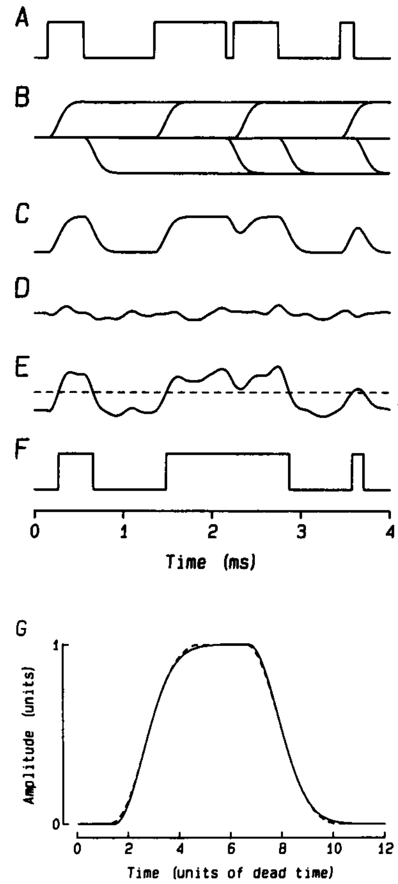

FIGURE 1 Simulating the true effects of filtering and noise on single-channel currents. (*A*) A single-channel current that would be recorded with unlimited time resolution and no noise. (*B*) Positive and negative filter step responses calculated with Eqs. 1 and 2, respectively, to the positive and negative current steps in *A*. (*C*) The filtered single-channel current is the sum of the step responses in *B*. (*D*) Noise filtered at the same level as the current in *C*. (*E*) The filtered and noisy single-channel current is the sum of the records in *C* and *D*. (*F*) The interval durations that would be measured when the record in *E* is analyzed with half-amplitude threshold detection. The dead time is 0.1 ms. (*G*) Plots of the filtered response to a square wave input into a four pole Bessel filter (24 dB/octave) (*dashed line*) and as calculated with Eqs. 1 and 2 (*solid line*).

simulated current record shown in Fig. 1 *E*. The filtering for the simulated current record and noise is selected to be identical to that used to analyze the experimental current record, and the time base for the filtered current in Fig. 1 *C* and noise in Fig. 1 *D* are the same so that they can be directly added.

Once the filtered and noisy current record in Fig. 1 *E* is generated, it is analyzed in the exact same manner with the exact same sample period as used to analyze the experimental current record. For this paper, current records have been analyzed with half-amplitude threshold detection (Colquhoun and Sigworth, 1983; McManus et al., 1987), but other detection methods could be used as long as experimental and simulated data are analyzed the same. Fig. 1 *F* shows the durations of the detected simulated intervals when the noisy and filtered data in Fig. 1 *E* are threshold analyzed. The interval durations (dwell-times) in Fig. 1 *F* are those that would be observed experimentally for a channel with the gating described in Fig. 1 *A*.

Generating the step-filter responses

To facilitate the analysis and to allow the same equations and simulation program to be used for all levels of filtering, the durations of simulated and experimental currents are expressed in units of dead time, defined as the true duration (the duration without filtering) of an interval that gives a half-amplitude response with filtering (Colquhoun and Sigworth, 1983). The time base for Fig. 1, *A–F*, is in milliseconds, and the dead time is 0.1 ms. The data in Fig. 1, *A–F*, can be expressed in units of dead time by relabeling the abscissa to range from 0 to 40 units of dead time.

When interval durations are expressed in units of dead time, the filter response for each channel opening, Y_o, and closing, Y_c, is calculated for a four-pole (24 dB/octave) Bessel filter with

$$Y_o = 1 - \exp[-C(t - T_o)^2]; \quad Y_o = 0 \text{ for } t < T_o \qquad (1)$$

$$Y_c = \exp\{-C(t - T_c)]^2\} - 1; \quad Y_c = 0 \text{ for } t < T_c, \qquad (2)$$

where time t is in units of dead time, T_o and T_c are the true times of channel opening and closing, respectively, in units of dead time, and $C = 0.6211$ (e.g., Colquhoun and Sigworth, 1983). For each event $Y_o(t)$ goes from 0 to 1, and $Y_c(t)$ goes from 0 to -1. Because $Y_c(t)$ always follows $Y_o(t)$ (the channel closes after opening), the resulting sum of the responses varies from 0 to 1. The constant C in Eqs. 1 and 2 was determined empirically to give a half-amplitude response for an interval with a true duration equal to the dead time. Fig. 1 *G* shows that the calculated filter response (Eqs. 1 and 2) to a square wave is very similar to the output of a four pole (24 dB/octave) Bessel filter with a square wave input. The filter used was an Ithaco 4302 (Ithaca Inc., Ithaca, NY) set in pulse mode. Although Eqs. 1 and 2 give a close approximation of the actual filter response, there are some differences, as the true filter response tends to overshoot and undershoot the calculated response slightly. Any potential error from these differences could be eliminated entirely by sampling the filter response of the actual experimental equipment (including filters) to a step-on and step-off response, and then using the digitized on- and off-responses in place of the calculated filter response in the simulation. This aproach would allow filters with properties that differ from four-pole Bessel filters to be used for the analysis.

Selecting the noise used in the simulation

For those channels which enter long-lasting shut states, long current records of baseline noise without channel activity could be obtained for use in the simulation. If the channel is too active to obtain a current record of the desired length without channel openings, then several shorter segments of noise could be used or noise could be obtained from a different patch without channel activity or from an artificial patch consisting of a resistor and capacitor. The filtering for these cases would be the same as for the experimental data, and the root-mean-square amplitude of the noise would be adjusted to match that of the closed channel noise in the experiment. Because the same noise record is used repeatedly in the simulation, it is important that it be as long as possible to be representative.

The analysis in this paper has been restricted to examples in which noise peaks in the absence of channel activity do not exceed threshold for event detection. Under these conditions it was found that estimates of the kinetic parameters could be sensitive to the characteristics of the noise used in the simulation, even if the root-mean-square values of the different noise records were the same. When the dead times were brief relative to the time constants of the fastest components in the distributions, so that most of the intervals were detected, then the estimated parameters were insensitive to the characteristics of the noise, provided the root-mean-square values were similar. With larger dead times and a smaller fraction of detected intervals, the estimated parameters became increasingly sensitive to the characteristics of the noise. The effects of using nonrepresentive noise in the simulation have not been systematically examined, however, because in most cases suitable noise should be obtainable from a different or artificial patch. In any case, the inclusion of noise in the simulation, even if not ideally representative, was found to allow better estimates of the kinetic parameters than if the effects of noise were ignored.

Reducing the time required for simulation

It is the step-response method of filtering (Eqs. 1 and 2, Fig. 1) together with the additional time-saving steps outlined below that makes simulation of single-channel data sufficiently fast so that the iterative simulation method can be used to determine kinetic parameters with affordable computers. Standard forms of digital filtering would, in general, be too slow for practical application of the method.

To further speed the simulation, the step filter responses are not calculated each time, but read from an array. For experiments in which the peak noise is less than the half-amplitude level, considerable time can be saved through special treatment of those intervals whose true durations are greater than four dead times. Only the rising and falling phases of these intervals are simulated (for a duration of four dead times each) and the excluded time is added in directly. Because the same array is used repeatedly for simulating the single-channel current, it is more efficient to first write the noise into the array and then sum in the filtered step responses. This negates the necessity of zeroing the array each time.

The simulated current is only generated at the times the current record is sampled. Thus, slower sampling rates can speed the simulation appreciably. For the findings presented in the Results section of this study the current records have been sampled at 20 times per dead time. In general, this is considerably faster than would be needed. Decreasing the sampling rate to five samples per dead time introduced no additional error for the two-state model and doubled the simulation rate. One or two samples per dead time might be sufficient for some applications. Even though the sampling rate for simulated data is always selected to be identical to that for the experimental data, errors could still arise if the sampling rate becomes too slow. First, events of brief duration that exceed threshold can be missed if a sample is not taken when the event is above threshold. Although the fraction of missed events would be the same for experimental and simulated data, the kinetic information contained in the additional missed events would be lost, decreasing the ability to estimate parameters. A second potential problem associated with slow sampling arises from the method used to simulate the current record. The durations of simulated intervals are treated as integral numbers of sampling periods by taking the integer of the true duration of the interval in sampling periods plus 0.5. Thus, intervals with true durations <0.5 sampling periods are excluded. For sampling rates greater than about two to five samples per dead time the excluded intervals would be sufficiently brief that, for most models, they would have little influence on the observed current levels, even when summing with previous responses near threshold. For sampling rates less than two samples per dead time, the numbers and durations of such ignored intervals would be greater so that errors could start to result, depending on the model.

Two-dimensional dwell-time distributions

To retain the correlation information during the analysis, the consecutive interval durations are binned into two-dimensional dwell-time distributions (Fredkin et al., 1985). One-dimensional distributions are not sufficient, and third or higher order distributions contain little additional information (Fredkin et al., 1985). For this paper we have used two-dimensional distributions of adjacent open and shut dwell times, but additional two-dimensional distributions of open-open, shut-shut, and open-shut dwell times, at a series of different lags could easily be included, with simultaneous fitting of all the different two-dimensional dwell-time distributions. Also, data under a variety of different experimental conditions could be simultaneously fitted to increase the ability to discriminate models (McManus and Magleby, 1986; Bauer et al., 1987; Ball and Sansom, 1989; Kienker, 1989).

The log-binning technique described in McManus et al. (1987) has been extended to generate the two-dimensional dwell-time distributions used for the fitting. In most cases the dwell-times have been binned at a resolution of 10 or 15 bins per log unit. Log-binning provides a convenient method to combine data to reduce stochastic fluctuation (especially important for the simulation method described in this paper). Log-binning can also greatly decrease the required time for maximum likelihood fitting (McManus et al., 1987) because all the events in each bin are treated simultaneously.

The two-dimensional log-binning is performed as follows. Consider a data record with consecutive open and shut dwell-times of the following durations: O1, S1, O2, S2, O3, S3. The intervals are binned (summed) into a two-dimensional distribution, with the durations of each open-shut pair of intervals determining the position on the two-dimensional (open dwell time versus shut dwell time) x-y grid. First, the pair of intervals O1 and S1 are binned, then the pair of intervals S1 and O2, then the pair O2 and S2, and this process is repeated for all pairs. This form of binning assumes microscopic reversibility, so that the relationship between the intervals is the same independent of whether they are analyzed forward or backward in time (e.g., Colquhoun and Hawkes, 1982).

A plot of the two-dimensional dwell-time distribution for intervals from the two-state model described by Scheme 1 in the Results is shown in Fig. 2. Intervals were generated by simulation, as in Fig. 1, to account for the true effects of filtering and noise. The detected open and shut interval pairs were then log-binned as indicated above. Plots of log-binned exponentially decaying data made without compensation for the changing bin size give a peak at the time constant of the exponential (Sigworth and Sine, 1987). The peak in Fig. 2 indicates the time constants of the open and shut dwell-time distributions. Two-dimensional distributions were fitted with the iterative simulation method to estimate errors in rate constants, as described in later sections and the Appendix.

When applying the method to actual experimental data, all the open-shut interval pairs and all the shut-open interval pairs would first be binned separately. If the resulting two-dimensional dwell-time distributions were found to be the same, then the data would be consistent with microscopic reversibility, and the open-shut and shut-open pairs could be combined into a single two-dimensional distribution. If the distributions were different, then the data would not be combined, but would be fitted separately and simultaneously to preserve this information.

Plotting one-dimensional dwell-time distributions

Although the errors associated with estimating rate constants by the iterative simulation method were obtained by fitting two-dimensional dwell-time distributions (example in Fig. 2), one-dimensional dwell-time distributions are plotted in the Results section. One-dimensional distributions allow direct comparisons with previous methods which have been based on such distributions. Details for binning and plotting one-dimensional distributions are given in Blatz and Magleby (1986*b*) and McManus et al. (1987). Additional examples and discussion of two-dimensional distributions are presented in a study describing their application to model discrimination (Magleby and Weiss, 1990*b*).

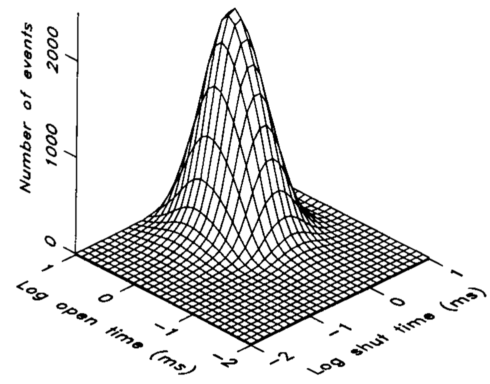

FIGURE 2 Two-dimensional dwell-time distributions of intervals generated by the two-state model described by Scheme 1. The interval durations used for the plot were determined by simulation (Fig. 1), with true filtering to give a dead time of 0.25 ms; the added noise had a standard deviation of 14% and a peak amplitude of 45% of the single-channel current amplitude. Each pair of detected open and shut intervals for 200,000 intervals was summed into bins, with the duration of the open interval locating the bin on the y axis and the duration of the shut interval locating the bin on the x axis. The z axis indicates the number of pairs in each bin. Log-binning is used so that bin width remains a constant fraction of bin midtime (McManus et al., 1987). The number of events in each bin is plotted directly without correction for the increase in bin area with increasing interval duration, similar to the transform used by Sigworth and Sine (1987) for one-dimensional distributions. The plot was generated by the program Surfer (Golden Software, Golden, CO) with the cubic spline option selected.

Searching for parameters using iterative simulation

The search routine changes the rate constants, typically one or more at a time, first in one direction, and then if there is no improvement in the likelihood, in the other direction. The changes in the rate constants are carried out to preserve microscopic reversibility, although this is not a requirement of the general method. If the likelihood is improved, then the changed rate constants are kept and new rates changed. The step sizes for the individual rates (or groups of rates to maintain microscopic reversibility) are auto ranging with the step size increasing on improvement and decreasing if there is no improvement. If a lower limit in step size is reached, the step size is reset to a specified larger size and the auto ranging continued from this point. An example of a search routine, Pattern Search, is given in Colquhoun (1971).

The search is typically started by simulating 100,000 intervals and by restricting the auto ranging step size to between ~5 and 50% of each parameter value. After 25–100 complete passes through all the parameters, the number of simulated events per distribution is increased by a factor of ~4 and the minimum step size decreased by a factor of ~2.

Such an increase in events and decrease in step size is repeated until any further changes in the likelihood become insignificant. If the step size is too small for the number of simulated events, then considerable time can be lost in the fitting process. Under these circumstances the change in likelihood from stepping one or more rates is typically less than the stochastic variation, and little progress is made. Because the two-dimensional dwell-time distribution predicted for any given set of rate constants is determined by simulation, there is some stochastic variability in the predicted response. Standard search routines are typically not designed to deal with such variability, with the result that they can become locked into a solution which is not the most likely one. This occurs when chance variability in the predicted response leads to an unusually favorable likelihood, and further changes in the rates using the standard search methods cannot better this likelihood. To circumvent this problem during the search, the best likelihood estimate is recalculated after each complete pass through all the rate constants by using the latest estimate of the most likely rate constants. The new best likelihood estimate will differ from the previous best estimate due to stochastic variation. The new estimate is then averaged (with 20% weighting) with the previous best estimate. If the previous estimate was unsually high due to chance alone, then the new weighted estimate is likely to be more representative.

Estimating errors in kinetic parameters

The magnitudes of the errors in estimating kinetic parameters will depend on the number of intervals in the fitted (experimental) data set, the number of simulated intervals used for the fitting, the level of filtering and noise, and the kinetic scheme. For this paper we have used large numbers of fitted and simulated intervals to greatly reduce errors which might arise from stochastic variation. Because the simulation method appears to be no more sensitive to stochastic variation in the fitted data than the matrix method described below (Magleby and Weiss, 1990*b*), the comparison of the two methods in this study reflects errors arising mainly from differences in the ability of the methods to account for the true effects of filtering and noise.

Simulating single-channel currents with an assumption of idealized filtering

To investigate the effects of an assumption of idealized filtering on the dwell-time distributions, the true currents, as in Fig. 1 *A*, were filtered by assuming that all events less than a specified duration, the dead time, were missed. For example, for a selected dead time of 0.1 ms, a sequence of open, shut, open ... interval durations before filtering with true durations of 0.5, 0.4, 0.09, 0.07, 0.08, 0.7, and 2 ms would become 0.5, 1.34, and 2 ms after idealized filtering. An example of such idealized filtering is shown in Fig. 3 *D* in the Results.

Estimating rate constants with an assumption of idealized filtering

The matrix method outlined in Blatz and Magleby (1986*a*) and McManus et al. (1987) has been used to estimate rate constants when the assumption of idealized filtering was used to correct for missed events. This method fits (one-dimensional) open and shut dwell-time distributions, and is based on the Q-matrix method of Colquhoun and Hawkes (1977, 1981) with the addition of an approximate correction for the effects of limited time resolution on missed events. The correction for limited time resolution assumes idealized filtering so that all events less than a specified duration, the dead time, are missed. For two-state models, the modified matrix method is equivalent to the semianalytical correction methods based on an assumption of idealized filtering presented in Colquhoun and Sigworth (1983) and Yeo et al. (1988). Rate constants were obtained with an assumption of idealized filtering so that they could be compared to those obtained by the iterative simulation method (detailed in the Results).

RESULTS

Single-channel intervals less than the dead time can be detected

Methods for the kinetic analysis of single-channel data typically assume that the current record is filtered in an idealized manner so that all events whose true durations are less than a specified duration, the dead time, go undetected (Colquhoun and Sigworth, 1983; Blatz and Magleby, 1986*a*; Ball and Sansom, 1988; Yeo et al., 1988; Milne et al., 1988). Fig. 3 shows that an assumption of idealized filtering can lead to error because events with durations less than the dead time can sum with the decaying responses from previous intervals and be detected. Fig. 3 *A* presents an example of a (true) single channel current record that would be recorded with

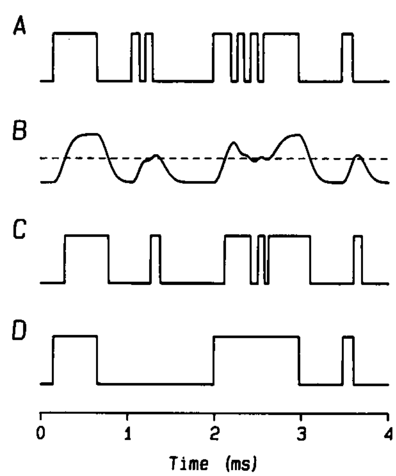

FIGURE 3 Intervals with true durations less than the dead time can be detected. (*A*) A single-channel current that would be recorded with unlimited time resolution and no noise. These are the true intervals. The intervals indicated by the brackets all have true durations <0.1 ms. (*B*) The single-channel current that would be observed when the true record in *A* is filtered to give a dead time of 0.1 ms. (*C*) The interval durations that would be measured when the record in *B* is analyzed with half-amplitude threshold detection. Notice that some of the intervals with true durations less than the dead time are detected. (*D*) The interval durations that would be measured from the record in *A* assuming that all intervals with true durations less than the dead time go undetected (assumption of idealized filtering). The difference between *C* and *D* gives the error introduced by an assumption of idealized filtering.

unlimited time resolution (no filtering). Fig. 3 *B* shows the record after filtering to give a dead time of 0.1 ms. The dashed line indicates the threshold for half-amplitude interval detection. The record in Fig. 3 *C* plots the intervals that would be detected with the half-amplitude threshold level from the filtered response in *B*. Fig. 3 *D* plots the intervals that would be detected from the true intervals in Fig. 3 *A* with an assumption of idealized filtering, so that all events with durations less than the dead time of 0.1 ms go undetected.

Several points are obvious from Fig. 3. True filtering together with threshold detection results in some intervals being missed entirely and the durations of other intervals being distorted (compare *A* with *C*). An assumption of idealized filtering to account for the effects of filtering can lead to additional missed events (compare *D* to *C*). Thus, analysis methods which assume idealized filtering, rather than true filtering, to correct for the actual effects of filtering can introduce considerable error. The following section investigates the nature of this error and shows that it can be made worse if the effects of noise are neglected.

Effects of filtering and noise on dwell-time distributions

Fig. 4 shows the effects of true filtering, idealized filtering, and noise on the open-and-shut dwell-time distributions for two different dead times for the two-state model given by Scheme 1,

$$C_2 \underset{1000/s}{\overset{2000/s}{\rightleftharpoons}} O_1, \tag{1}$$

where C_2 is the closed state and O_1 is the open state. The date for the distributions was obtained by simulation, as in Fig. 1, *A–F*. When added, the noise had a SD of 14%, and a peak amplitude of 45% of the single-channel amplitude. Thus, in the absence of channel activity the noise would not be detected as false events.

The continuous lines in Fig. 4 indicate the dwell-time distributions that would be observed for Scheme 1 when the true effects of filtering and noise are taken into account. The dashed lines in Fig. 4 indicate the distributions that would be observed if there were true filtering, but no noise. For Scheme 1 noise had little effect for a dead time of 0.25 ms, but decreased the durations of the detected open times for a dead time of 1.0 ms, as indicated by the faster decay of the open distributions. This decrease in detected open times results because more of the brief events are detected in the presence of noise (McManus et al., 1987).

The dotted lines in Fig. 4 indicate the distribution that would be observed with an assumption of idealized filtering and no noise. The difference between the continuous and dotted lines then gives a measure of the error associated with an assumption of idealized filtering and no noise. For a dead time of 0.25 ms (Fig. 4, *A* and *B*), the errors associated with an assumption of idealized filtering and no noise are small for dwell-times greater than twice the dead time. For times less than twice the dead time, however, large errors result with an assumption of idealized filtering. This is the case because for idealized filtering there are no intervals with durations less than the dead time, yet with true filtering a large fraction of the intervals can have durations less than the dead time due to the shortening of the measured durations of brief intervals, and because intervals with true durations less than the dead time can be detected with true filtering. To avoid this problem, methods which assume idealized filtering typically exclude from the fitting all intervals with dwell times less than twice the dead time, but such an exclusion can eliminate the data needed to define fast decaying components.

When the dead time is 1 ms (Fig. 4, *C* and *D*) the errors are substantial at all times, so that channel analysis techniques which assume idealized filtering and no noise would no longer be applicable. Furthermore, because much of the error arises from the assumption of idealized filtering, low noise data would not be sufficient to prevent error.

The fast (phantom) exponential component that arises as a consequence of missed events (Roux and Sauve, 1985; Blatz and Magleby, 1986*a*) is especially apparent at brief times in the distributions with idealized filtering in Fig. 4, *C* and *D* (*dotted lines*). This phantom component would also be present with true filtering, but is masked by the shortening of the brief intervals that occurs with true filtering.

The levels of filtering and resulting dead times for Fig. 4 were selected to be consistent with those usually used for channels of small conductance (Blatz and Magleby, 1986*c*) and for channels in lipid bilayers where the filtering is greater (Moczydlowski and Latorre, 1983). Under these conditions, fast exponential components are not detected so that the fastest rate constants for derived kinetic models are also slow, as in the case for Scheme 1.

With large conductance channels, the open channel current is greater so that less filtering is required for the analysis. Under these conditions dead times of 25–100 μs are typical, and the estimated rate constants for the kinetic schemes are faster because faster components in the data are detected (Colquhoun and Sakmann, 1985; Blatz and Magleby, 1986*b*). The results in Fig. 4 are directly applicable to data with shorter dead times provided that the interval durations for the plotted distributions are rescaled. With closing and opening rate con-

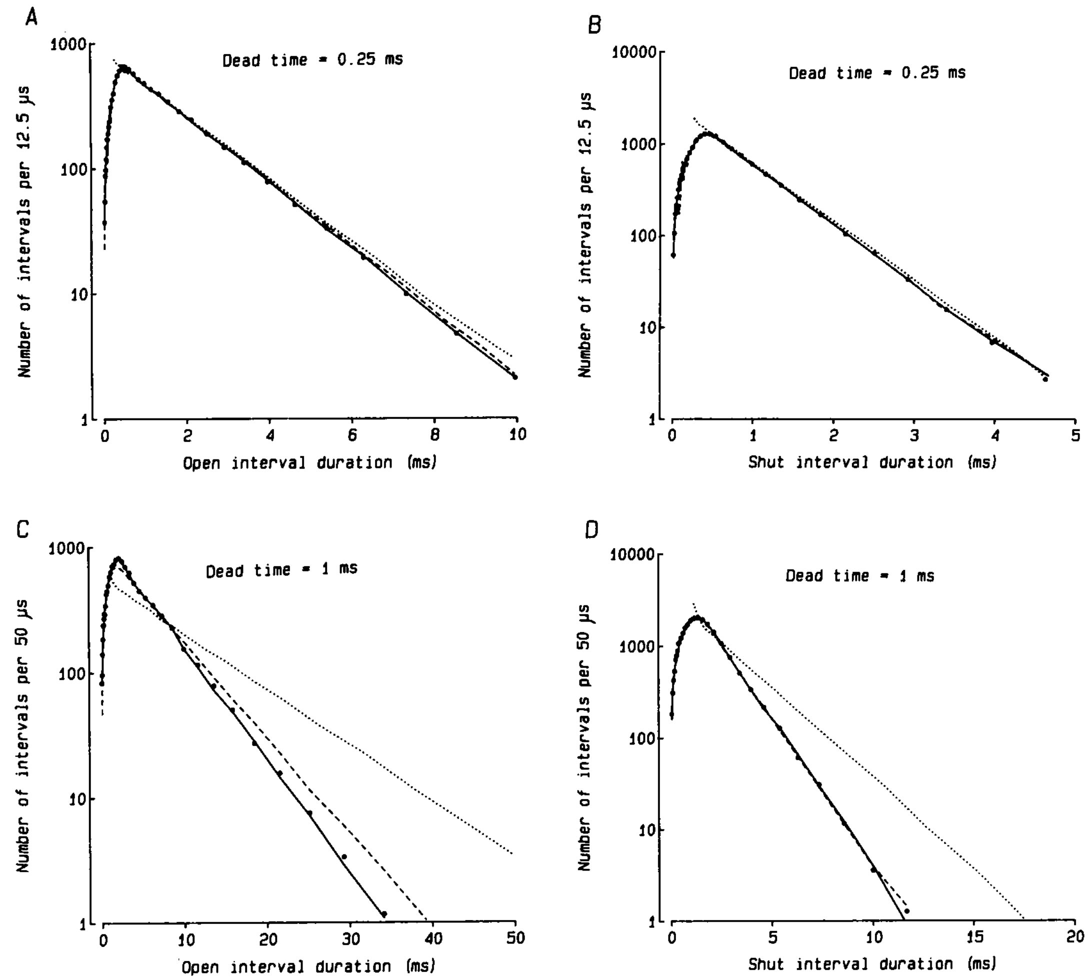

FIGURE 4 Open and shut dwell-time distributions for Scheme 1 with filtering to give dead times of 0.25 ms (*A* and *B*) and 1 ms (*C* and *D*). The continuous lines are the distributions that would be observed with true filtering and noise. (The noise had a standard deviation of 14% and a peak amplitude of 45% of the single-channel current amplitude.) The dashed lines are with true filtering and no noise. The dotted lines are with an assumption of idealized filtering. The solid symbols are the predicted distributions for the rate constants determined by the iterative simulation method. The distributions, each of which contains 100,000 events, were determined by simulation.

stants of 5,000 and 10,000/s for Scheme 1, the distributions observed with dead times of 50 and 200 μs are the same as those in Fig. 4, *A–B* and *C–D*, respectively, but with the interval durations on the abscissa divided by five. With rate constants of 10,000 and 20,000/s for Scheme 1, the distributions observed with dead times of 25 and 100 μs are the same as those in Fig. 4, *A–B* and *C–D*, respectively, but with the interval durations on the abscissa divided by ten.

Thus, the results in Fig. 4 and those obtained by scaling the abscissa in Fig. 4 for shorter dead times and faster rate constants show the error in the distributions that can arise when an assumption of idealized filtering and no noise is used to account for the true effects of filtering and noise. Analysis methods which assume idealized filtering and no noise will translate this error into errors in the estimated parameters. Furthermore, even for favorable situations in which the dead time is small compared to the mean lifetimes of the states, data less than about twice the dead time is still in error and must be excluded. What is needed, then, is a general method to estimate kinetic parameters which takes into account the true effects of filtering and noise and which uses all the data in the distributions.

The following sections demonstrate that a method based on the simulation of single-channel currents can provide such a general method. Simulation is used because there are no known general analytical or matrix methods which can account for the true effects of filtering and noise on missed events.

Estimating kinetic rate constants by iterative simulation

Fig. 5 outlines the method we have used to estimate kinetic rate constants by iterative simulation of single-channel currents. The first step is to analyze the experimental single-channel current record with half-amplitude threshold detection and bin the measured durations into two-dimensional (joint probability) dwell-time distributions. Such two-dimensional dwell-time distributions retain the useful kinetic information in the single-channel current record (Fredkin et al., 1985). An artificial single-channel current record is then obtained by simulating the proposed gating mechanism. The simulation is carried out with the same filtering and noise as that for the experimental single-channel current record, so that any effects of filtering and noise will be the same for both experimental and simulated data.

The simulated current record is then analyzed in the exact same manner as used for the experimental current record to obtain the predicted (simulated) two-dimensional dwell-time distribution. A likelihood comparison of the predicted and experimental distributions then gives the probability that the experimental data were generated by the given kinetic scheme and rate constants. A search routine changes the rates and the process is repeated as necessary to find the rates which maximize the likelihood. The details for the maximum likelihood search are in the Appendix, and the details for the other steps in Fig. 5 are in the Methods.

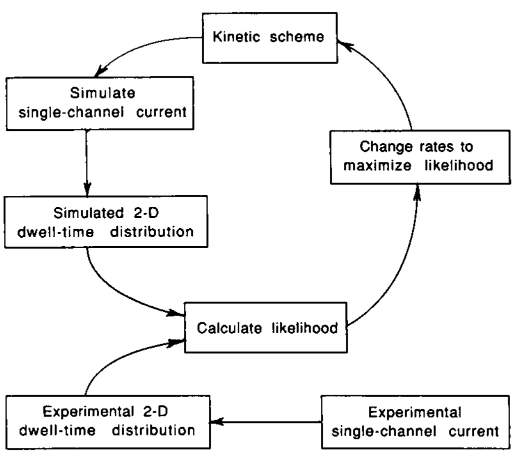

FIGURE 5 Iterative simulation method to estimate kinetic parameters from single-channel data. The first step is to analyze the experimental single-channel current record using half-amplitude threshold detection to measure interval durations. The experimental intervals are then binned into a two-dimensional dwell-time distribution. Then, starting wtih an assumed kinetic scheme and parameters, a filtered and noisy single-channel current is simulated as described in Fig. 1. The simulated current is then analyzed in the exact same manner as used to analyze the experimental data to obtain the simulated two-dimensional dwell-time distribution. A direct bin by bin comparison between the simulated and experimental two-dimensional dwell-time distributions (Eq. A3 in Appendix) is used to calculate the likelihood that the experimental data are generated by the assumed kinetic scheme. The process is then repeated in an iterative manner using a search routine to find the parameters which maximize the likelihood.

To discriminate among different gating mechanisms the iterative process is then repeated for each kinetic scheme. The examined schemes are than ranked and the significance of the ranking determined using criteria which take both the likelihood and number of free parameters into account (Rao, 1973; Horn and Lange, 1983; Horn and Vandenberg, 1984; Horn, 1987; McManus et al., 1988; Ball and Sansom, 1989).

Because the analysis method presented in Fig. 5 treats experimental and simulated data identically, any errors from noise, filtering, missed events, sampling, and binning (errors detailed in McManus et al., 1987) are of little consequence because they are essentially identical for both simulated and experimental data, and cancel out.

Errors in estimating rate constants by iterative simulation

To assess the ability of the iterative simulation method described in Fig. 5 to estimate rate constants, we first examined whether this method could estimate rates for the two-state model described by Scheme 1. Single-channel currents were generated for this scheme with various levels of filtering to yield data at different dead times and with noise. These single-channel currents were then treated as if they were experimental data; the interval durations were measured with half-amplitude threshold analysis and two-dimensional dwell-time distributions of adjacent open and shut dwell-times constructed. The iterative simulation method was then used to estimate rates from these two-dimensional dwell-time distributions. For this study large numbers of fitted and simulated events were used to reduce errors from stochastic variation, so that errors which might arise from the effects of filtering and noise would be more apparent.

Fig. 6 plots estimates of the opening and closing rates for Scheme 1 for the fitting of 200,000 intervals at each dead time by using 200,000 simulated intervals for each iteration. Under these conditions errors in estimating the rate constants by iterative simulation (solid symbols and solid line) were <6% of the true rates (dotted line) over a wide range of filtering to give dead times ranging from 0 to 3 ms. The SD bars indicate the uncertainty in the estimates, and were obtained by repeating the fitting with widely different starting parameters.

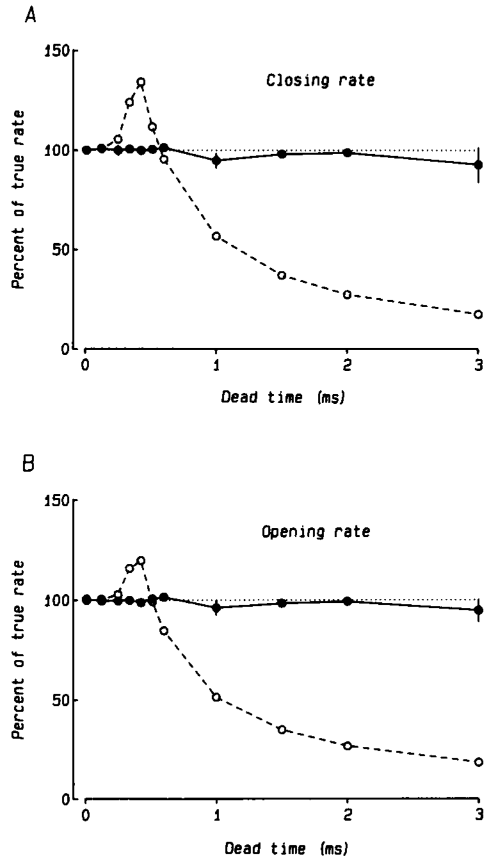

FIGURE 6 Estimating rate constants for Scheme 1 from current records with different levels of filtering to give dead times of 0–3 ms. The estimated rates are plotted as the percent of the true closing rate of 1,000/s in *A* and of the true opening rate of 2,000/s in *B*. The iterative simulation method (*solid circles: bars indicate the SD*) gave consistently better estimates of the rate constants than a fitting method based on the assumption of idealized filtering and no noise (*open circles*). Plots of the data that were fitted to estimate rate constants for a 1 ms dead time are given by the distributions described by the continuous lines in Fig. 3. Fitting with an assumption of idealized filtering was started at twice the dead time to exclude the distorted brief intervals.

Errors in estimating rate constants with an assumption of idealized filtering

The dashed lines in Fig. 6 plot errors in estimating rate constants for Scheme 1 using an assumption of idealized filtering and no noise, similar to the assumptions used in the semianalytical and matrix methods described in Colquhoun and Sigworth (1983, Eqs. 79 and 80), Blatz and Magleby (1986*a*), and Yeo et al. (1988). For brief dead times the rate constants were overestimated and for longer dead times they were grossly underestimated. For example, for dead times of 0.425 and 3 ms, the estimated rate constants for channel closing were 1,340 and 170/s, a 34% overestimate and sixfold underestimate, respectively, of the true rate constant of 1,000/s. The over- and underestimate of the rate constants with an assumption of idealized filtering and no noise reflects the complex effects of true filtering, noise and dead time on detecting intervals with 50% threshold detection.

The increased errors for the assumption of no noise and idealized filtering result because this assumption leads to incorrect (biased) estimates of the rate constants. The increased errors are not a result of stochastic variation in the fitted data, as such variation for a two-state model described with 200,000 intervals is negligible. This can be seen from Fig. 4, *C* and *D*, which plots data for a dead time of 1 ms. The open and shut distributions that were fitted are indicated by the solid lines, and are well defined. The distributions that would be predicted with an assumption of idealized filtering with the correct rate constants are indicated by the dotted lines, and are clearly different. Thus, with an assumption of idealized filtering incorrect rate constants (plotted in Fig. 6) are required to fit the correct distribution. In contrast, the iterative simulation method predicts both the correct rate constants (Fig. 6) and the correct distribution (*solid symbols* in Fig. 4).

For the analysis in Fig. 6 the closing and opening rate constants for Scheme 1 were 1,000/s and 2,000/s. For faster rates of 5,000 and 10,000/s the errors associated with the two methods are given by Fig. 6, but with the values of dead time on the abscissa divided by five. For rates of 10,000 and 20,000/s the values of dead time on the abscissa in Fig. 6 are divided by 10. Thus, with the faster rate constants, the errors associated with the assumption of idealized filtering and no noise used by the semianalytical and matrix methods can become significant for dead times as brief as 25–50 μs. For even faster rate constants, errors with an assumption of idealized filtering and no noise could occur at increasingly shorter dead times. If both rates are equally fast, then the errors are greater; when only one rate is fast then the errors can be considerably less or insignificant. The semianalytical and matrix methods did give highly repeatable estimates of rates, but repeatability is of little value if the estimates are incorrect.

The results in Fig. 6 show then, that the iterative simulation method, by accounting for the true effects of filtering and noise, gives consistently better estimates of the rate constants for Scheme 1 than methods based on assumptions of idealized filtering and no noise.

Excluding false solutions arising from an assumption of idealized filtering

Because semianalytical and matrix methods can be orders of magnitude faster than the iterative simulation method, it seems that such methods might be preferred for those conditions where errors introduced by the assumption of idealized filtering and no noise are small. Unfortunately, the assumption of idealized filtering used in the semianalytical methods can lead to another type of error not shown in Fig. 6. With an assumption of idealized filtering, two solutions of identical likelihood are obtained for two-state models (Colquhoun and Sigworth, 1983; Blatz and Magleby, 1986*a*; Yeo et al., 1988; Milne et al., 1989) as well as for more complex models (Blatz and Magleby, 1986*b*). For example, the true rates for Scheme 1 are 1,000 and 2,000/s for the closing and opening rates, respectively. When data from this scheme, filtered to have a dead time of 0.25 ms, were fitted with the semianalytical method, two solutions were obtained: a slower 'correct' solution of 1,055 and 2,058/s, and a faster incorrect solution of 4,568 and 6,931/s. It is the errors associated with the 'correct' solutions that are plotted in Fig. 6 as open circles.

Which of the two solutions given by the semianalytical method is the correct one (it is not always the slower one, Yeo et al., 1988), can, in principle, be determined by reanalyzing the original current record with a different level of filtering, (Colquhoun and Sigworth, 1983; Blatz and Magleby, 1986*b*; Yeo et al. 1988). It would be desireable, however, if such additional analysis, which can double the time required to analyze an experiment, were not necessary.

In contrast to the two solutions found with an assumption of idealized filtering, only the correct solution was found by the iterative simulation method. The reason for this is shown in Fig. 7. Fig. 7 *A* plots the open dwell-time distributions for the two solutions for Scheme 1 predicted with an assumption of idealized filtering. The continuous line is the predicted distribution for the correct solution (rate constants of 1,058 and 2,055/s), and the dotted line is the predicted distribution for the incorrect solution (rate constants of 4,568 and 6,931/s). With idealized filtering the correct solution cannot be distinguished from the incorrect solution because the predicted distributions are almost identical.

Fig. 7 *B* plots the open dwell-time distributions that would actually be observed for the correct and incorrect solutions if the true effects of filtering are taken into account. The distribution for the incorrect solution (*dotted line*) is clearly in error, decaying much faster than the distribution for the correct solution (*continuous line*). Because of this error, the incorrect solution found with an assumption of idealized filtering by the semianalytical method is not a solution for the iterative simulation method.

In theory, methods based on an assumption of idealized filtering might be able to distinguish the correct from the incorrect solution using the slight difference (apparent in

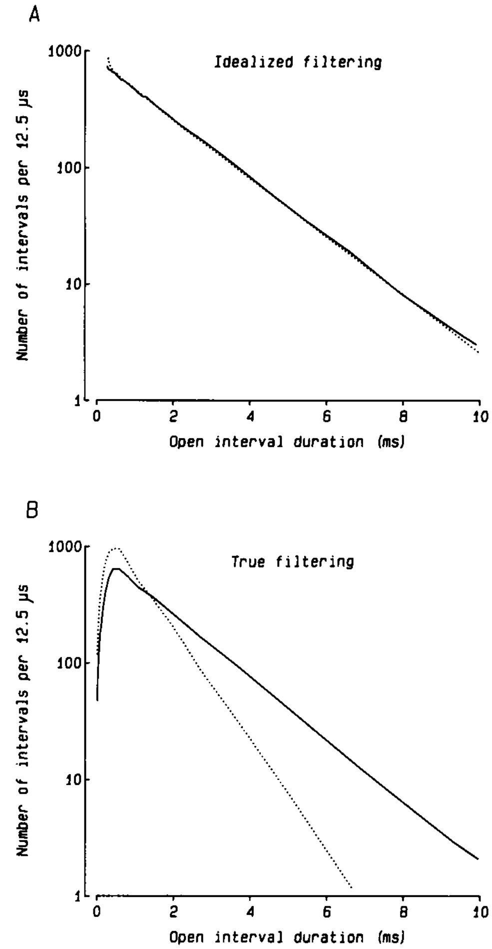

FIGURE 7 An assumption of idealized filtering leads to two solutions for Scheme 1, whereas there is only one solution when the true effects of filtering are taken into account. (*A*) The open dwell-time distributions for the two solutions for an assumption of idealized filtering and a dead time of 0.25 ms. The continuous line plots the open distribution for the correct solution of 1,055/s and 2,058/s and the dotted line plots the open distribution for the incorrect solution of 4,568/s and 6,931/s for the closing and opening rates, respectively. The distributions are similar except at very brief times. (*B*) The open dwell-time distributions that would actually be observed for the correct (*continuous line*) and incorrect (*dotted line*) solutions when the true effects of filtering are taken into account. With true filtering, the distribution for the incorrect solution is clearly in error. Hence, there is only one solution for Scheme 1 by the iterative simulation method. The plotted distributions of 100,000 events were determined by simulation.

Fig. 7 *A*) in the distributions at very brief times (Yeo et al., 1988). In practice, however, the predicted distributions for an assumption of idealized filtering are so different at brief times from the distributions that would actually be observed experimentally (cf. Fig. 7 *A* to the solid line in Fig. 7 *B*), that a direct distinction would be difficult.

Our finding of only one solution for two-state models by the iterative simulation method does not, of course, prove that a unique solution will always be found for two-state models with this method. We suspect, however, that this is likely to be the case because the iterative simulation fits the entire distribution without any simplifying assumptions as to its form. An incorrect set of rate constants for a two-state model might be expected to alter some part of the distribution when the true effects of filtering are taken into account.

This section shows, then, that determining rates by the iterative simulation method can exclude the incorrect solution which results from an assumption of idealized filtering. This advantage of the iterative simulation method should also apply to models with more than two states, as more complex models can also produce incorrect solutions resulting specifically from an assumption of idealized filtering (Blatz and Magleby, 1986*b*).

Estimating kinetic rates by iterative simulation for a three-state model

To explore whether kinetic parameters can be determined by iterative simulation for more complex models than the two-state model considered in the previous section, three- and five-state models were examined. The three-state model was described by

$$C \underset{200}{\overset{100}{\rightleftharpoons}} C \underset{500}{\overset{1{,}000}{\rightleftharpoons}} O, \qquad (2)$$

with filtering to give a dead time of 1.6 ms and noise the same as described previously for the two-state model. Data for fitting were generated for Scheme 2 by simulation with noise as described for Scheme 1; 10^6 intervals were generated for fitting to reduce stochastic variation. The data were then log-binned and the same 10^6 intervals were fitted by a modified Q-matrix method which assumes idealized filtering and no noise (Blatz and Magleby, 1986*a*) and by iterative simulation. When fitting by iterative simulation, 10^6 intervals were simulated for each iteration.

The rate constants estimated by iterative simulation were almost identical to the true rate constants, with average errors for the four rate constants of 2.3 ± 1.7% (range, 0.17–4.3%). If the iterative simulation fitting were performed with an assumption of no noise, even though there was noise in the original data, then the errors in estimates of the four rate constants were 9.2 ± 4.3% with a range of 4.8 to 26%. Thus, it is important to take the effects of noise into account. Determining rates by the matrix method which assumes idealized filtering and no noise gave errors of 40 ± 23% (range, 12–69%) for the 'correct' solution and larger errors for the incorrect (second) solution.

The increased errors when the effects of noise were excluded from the iterative simulation method and the increased errors with the matrix method arose because the estimated rate constants under these conditions were incorrect (biased). The increased errors were not a result of stochastic variation because of the large numbers of fitted and simulated intervals; the absence of noise alone or the assumption of no noise and idealized filtering clearly changed the distributions, as was the case for the distributions shown in Fig. 4 for the two-state model.

Thus, iterative simulation gave considerably better estimates of the rate constants for the three-state model described by Scheme 2 with a dead time of 1.6 ms, than a matrix method which assumes idealized filtering and no noise. Whereas 1.6 ms is a large dead time for large conductance channels, filtering to produce a dead time of this magnitude is not unusual for smaller conductance channels. If the rate constnats for Scheme 2 were 10- or 20-fold higher, then the same magnitude errors would occur for dead times of 0.16 or 0.08 ms, respectively.

Estimating kinetic rates by iterative simulation for a five-state model

We also examined the ability of the iterative simulation method to determine rate constants for the five-state model indicated by Scheme 3 with rate constants (per second) of: 1–3, 322; 2–4, 2860; 3–1, 3950; 3–4, 600; 4–2, 120; 4–3, 285; 4–5, 180, and 5–4, 34.

$$\begin{array}{ccccc} C_5 & \rightleftharpoons & C_4 & \rightleftharpoons & C_3 \\ & & \upharpoonleft\downharpoonright & & \upharpoonleft\downharpoonright \\ & & O_2 & & O_1 \end{array} \qquad (3)$$

Data were generated for Scheme 3 with true filtering to give dead times of either 0.15 or 0.5 ms, and with noise the same as described for Scheme 1; 10^6 intervals to be fitted were generated for each dead time to reduce stochastic variation. The data were then log-binned and the same intervals were fitted by the iterative simulation method and by a modified Q-matrix method which assumes idealized filtering and no noise (Blatz and Magleby, 1986*a*). For fitting with the iterative simulation method, 10^6 intervals were simulated for each iteration. Scheme 3 was chosen because it can approximate the activity of the large conductance Ca-activated K channel for moderate time resolution and a single concentration of Ca^{2+} (Magleby and Pallotta, 1983).

With a dead time of 0.15 ms, the iterative simulation method found a single best solution for Scheme 3 and estimated the eight rate constants with an average error of 2.3 ± 2.4% (range, 0.25–7.7%).

With a dead time of 0.15 ms the semianalytical method gave four solutions for Scheme 3, all with identical likelihood. The correct solution was easily identified because the kinetic scheme was known. For one of the incorrect (but equally likely) solutions, the rate constants were changed so that the longest lifetime open state was O_2 instead of O_1 and the shortest lifetime shut state was C_2 rather than C_1. This equally likely reversed solution demonstrates that fitting procedures based on one dimensional distributions often cannot define unique rate constants (Fredkin et al., 1985; Blatz and Magleby, 1986*b*). The other two incorrect (but equally likely) solutions for the semianalytical method arose from the assumption of idealized filtering. Because the correct solution could be identified from the kinetic scheme used to simulate the data (a luxury not available for experimental data), the incorrect solutions for the semianalytical method will not be considered further. The average error in estimating the eight rate constants with the semianalytical method was 2.02 ± 2.3% (range, 0.1–7.1%). (This error is greater than in Blatz and Magleby (1986*a*) where idealized filtering was used to filter the data being fitted and there was no noise. In fact, errors associated with corrections for missed events have typically been underestimated in the literature because the true effects of filtering and noise have been ignored.)

Thus, for Scheme 3 with a brief dead time (0.15 ms) relative to the fastest time constants, the matrix method and the iterative simulation method have similar error, provided that the correct solution can be identified for the semianalytical method. The iterative simulation method has the advantage, however, of giving a single (correct) solution to Scheme 3.

When the dead time was increased to 0.5 ms the iterative simulation method still found a single solution, estimating the rate constants with an average error of 2.0 ± 2.2% (range, 0.1–5.7%). In contrast, the semianalytical method still found four equally likely solutions, and estimated the rate constants for the correct solution with an average error of 16 ± 21% (range, 1.5–63%). Thus, with the greater dead time, the iterative simulation method has two advantages: there is only one solution, and the average error in estimating the rates is eight times less than the semianalytical method.

DISCUSSION

The advantages and disadvantages of estimating kinetic parameters from single-channel data by the iterative simulation method will be considered.

Advantages of the iterative simulation method

(*a*) The iterative simulation method is easy to understand. There are no complex matrix manipulations required to estimate distributions nor are there equations derived with simplifying assumptions to correct for missed events. A single-channel current is generated by simulating the proposed channel gating mechanism. This simulated current is then analyzed in the exact same way used to analyze the experimental single-channel current to obtain a two-dimensional dwell-time distribution. The simulated and experimental distributions are then compared with a direct bin by bin likelihood calculation (Eq. A3 in Appendix), and the entire process repeated until the maximum likelihood estimates of the parameters are found (Fig. 5).

(*b*) The iterative simulation method accounts for the true effects of filtering and noise on interval durations and missed events. Consequently, the iterative simulation method gives consistently better estimates of the rate constants (Fig. 6, *solid line*) than standard methods of analysis which assume idealized filtering and no noise (Fig. 6, *dashed line*).

(*c*) The iterative simulation method allows all the detected intervals to be used in the fitting process, including those of brief duration which are highly distorted by the filtering. All intervals can be used because any distortions of the interval durations due to filtering, noise, missed events, sampling, binning and other analysis errors are the same for both experimental and simulated data, and cancel out.

In contrast, methods which assume idealized filtering (defined in Fig. 3) typically exclude brief intervals (those with durations less than two dead times). It would, of course, be possible in these methods to correct the durations of each of the brief intervals in the experimental data before comparison to the predicted distributions (equations in Colquhoun and Sigworth, 1983), but such correction can lead to additional error for two reasons. First, the correction does not take into account the effects of noise, which can lead to an excess detection of the brief intervals (McManus et al., 1987). Second, it is the brief intervals that give rise to the fast exponential component evident in Figs. 4 and 7 *A* (*dotted lines*). If the assumption is then made, as is the case for most analysis methods which assume idealized filtering, that the fast exponential component is insignificant, then including the brief intervals in the fitting can lead to distorted parameters and/or an erroneous conclusion of an additional kinetic state.

(*d*) Because it takes into account the true effects of filtering, the iterative simulation method automatically excludes the false solutions (Fig. 7; Colquhoun and Hawkes, 1983; Blatz and Magleby, 1986*a*; Yeo et al., 1988) which arise with methods which assume idealized filtering.

(*e*) The two-dimensional dwell-time distributions used in the iterative simulation method contain the important correlation information among intervals (Fredkin et al., 1985). The correlation information gives the iterative simulation method increased ability over methods which use one-dimensional dwell-time distributions to both find the correct set of rate constants for any given model, as shown for the examined five-state model, and to identify the correct model, as shown in Magleby and Weiss, 1990*b*. Analysis methods restricted to one-dimensional distributions often cannot identify either the correct set of rate constants or the correct model (Blatz and Magleby, 1986*b*; Magleby and Weiss, 1990*b*).

(*f*) The iterative simulation method can be used to study channel kinetics with data obtained from multichannel patches. For example, for a patch with three overlapping active channels the predicted two-dimensional dwell-time distributions at each of three current levels could be calculated by simulation and compared to the experimental two-dimensional dwell-time distributions at the same current levels. Data from multichannel patches in which the channels desensitize have typically been analyzed by studying isolated bursts of activity, each generated by a single-active channel (Colquhoun and Sakmann, 1985). The iterative simulation method could also be applied to such data by comparing 2-D dwell-time distributions of intervals during bursts of experimental data with 2-D dwell-time distributions of intervals during bursts of simulated data. The minimum shut interval used to define bursts would be the same in each case.

(*g*) The iterative simulation method makes no assumptions as to the form of the predicted dwell-time distributions and places no restrictions on the proposed gating mechanism, except that the channel typically opens to a single conductance level. Thus, the iterative simulation method can be applied to non-Markov as well as to Markov gating mechanisms, and thermodynamic equilibrium is not a requirement. The iterative simulation method also provides a framework for investigations of mechanisms which extend beyond the concept of kinetic states. For example, proposed conformational changes and the associated gating might be simulated for direct comparison to experimental data.

(*h*) The iterative simulation method is well suited for limited amounts of data because the fitting is based on maximum likelihood. The likelihood that each experimental pair of intervals is drawn from the predicted (simulated) distribution is calculated independently of all the other experimental pairs of intervals. Consequently, there is no penalty for empty bins, nor is there a requirement that a minimum number of experimental events be in each bin, as is the case for least squares fitting. For these reasons and because of the advantages listed in *a*–*h*, the iterative simulation method might be expected to give a better estimate of parameters and gating mechanism for limited amounts of experimental data than most other methods. (An example of identifying the correct model with only 2,000 events is given in Magleby and Weiss, 1990*b*). Larger amounts of data will, of course, further increase the ability to estimate parameters and discriminate among gating mechanisms.

(*i*) The iterative simulation method is also well suited for very large amounts of data. The time required for fitting is almost independent of the number of experimental data points because the experimental data are combined by log-binning (McManus et al., 1987).

Thus, the iterative simulation method is a general method, as it can be used for different types of gating mechanisms while accounting for the true effects of filtering and noise on interval durations and missed events.

Disadvantages of the iterative stimulation method

(*a*) A disadvantage of the iterative simulation method, as presented, is that it is not directly applicable to channels which frequently enter subconductance states. However, it is likely that the method could be expanded to study such channels by including multiple threshold levels in the analysis of both the experimental and simulated data. Two-dimensional distributions of the conductance levels of adjacent intervals as well as various two-dimensional distributions of adjacent dwell times contingent on the various conductance levels could then be obtained from the experimental and simulated data used in the fitting. Furthermore, such an automated analysis of channels with subconductance levels might be preferred, as it would eliminate the subjective decisions inherent in visual analysis as to whether a transition to an apparent subconductance level results from an actual subconductance state or from filtering and missed events.

(*b*) A second disadvantage of the iterative simulation method is the slowness of the fitting procedure. Solutions for a two-state model typically took 24 h of continuous calculations with a XP-accelerator board (Cheshire Engineering Corp.), which has about twice the effective power of a 20 mHz 386 computer with an 80387 math coprocessor. Solutions for three- and five-state models took 1–4 d, and the time required for more complicated models, including the simultaneous fitting of data obtained under several different experimental conditions, such as multiple voltages and or concentrations, would be considerably greater.

Fortunately, the problem of fitting time is resolvable. Computers with 3–5 times the power of the XP-accelerator board are available now as modest priced workstations, and in 1–2 yr the power should be two to four times this. Alternatively, the iterative simulation is an ideal computation for a parallel processing computer. Each processor, supplied with a different random number seed, can simulate data independently of the others. The necessary computing time then becomes (almost) inversely related to the number of processors. We have assembled such a parallel processing computer with 40 processing units (Inmos T800, available from Definicon Systems, Newbury Park, CA) that has an effective computing power of about 100–200 million instructions per second (MIPS). Such computing power should decrease computation times 20-fold over a single XP-accelerator board, bringing the practical application of the iterative simulation method within reach for complex models. Even with this computing power the iterative simulation method will still be slow compared to nonsimulation methods, but this may be an acceptable penalty to reduce errors and identify the most likely models.

(*c*) Another disadvantage of the iterative simulation method is the variation introduced in the estimates of the rate constants by the simulation process itself. Even with large numbers of simulated events (10^6) there is still variation in the predicted response due to the fact that the interaction between the simulated current and added noise is different for each fitting because of the stochastic variation in the predicted interval durations. The consequence of this variation is twofold: first, repeated estimates of the rate constants can vary, and second, exponential components which have small effects on the likelihood compared to the effects of stochastic variation will be unresolved or poorly resolved.

The inability to obtain highly repeatable estimates of the rates by iterative simulation does not necessarily favor methods which assume idealized filtering and no noise, as these assumptions typically lead to greater errors than those introduced by the variation in the iterative simulation method (Fig. 6). However, matrix analysis methods may be necessary to resolve those components that have small effects on the likelihood.

(*d*) The iterative simulation method is not a full likelihood method even though it uses maximum likelihood fitting to compare the simulated and experimental two-dimensional distributions. Full maximum likelihood fitting would require maximizing the likelihood that the actual observed sequence of open-and-shut intervals occurred, as in the landmark method of Horn and Lange (1983) and in the method by Ball and Sansom (1989). These full likelihood methods might also be more useful for nonstationary data, but have not yet been expanded to account for the true effects of filtering and noise on missed events.

Conclusion

Given sufficient computer power, the iterative simulation method should provide a powerful technique with which to study single-channel gating mechanisms.

APPENDIX: MAXIMUM LIKELIHOOD FITTING OF TWO-DIMENSIONAL DWELL-TIME DISTRIBUTIONS

The appendix details the maximum likelihood fitting procedure used to estimate kinetic rate constants. The objective is to develop a general method that is not restricted as to the form of the dwell-time distributions or the level of noise and filtering. To this end we have used direct likelihood comparisons of simulated and experimental two-dimensional dwell-time distributions, without any intervening analytical approximations. In developing a maximum likelihood method for estimating rate constants from two-dimensional dwell-time distributions (example in Fig. 2), we have extended the approach presented by Colquhoun and Sigworth (1983) for fitting one-dimensional dwell-time distributions with sums of exponentials.

The parameters to be estimated are the rate constants (or any other factors which can affect gating) for a given kinetic scheme. The parameters have values of $\theta 1, \theta 2, \theta 3 \ldots$ and are collectively designated by the symbol θ. The experimental data are represented as a two-dimensional distribution of open-shut dwell times (see Methods). The experimental data are thus fixed and consist of the number of events in each of the bins in the distribution: $D(t_{O1}, t_{C1})$, $D(t_{O1}, t_{C2}) \ldots D(t_{On}, t_{Cn})$, where $t_{O1} \ldots t_{On}$, and $t_{C1} \ldots t_{Cn}$ represent the open and shut mid–dwell times of each bin, respectively.

For a given kinetic scheme and rate constants θ the predicted 2-D dwell-time distribution is calculated by simulating and binning 10^5 to 10^8 events (see Methods). The probability $P(t_{Oi}, t_{Cj})$ of observing an open-shut dwell-time pair in the bind with mid–dwell times of t_{Oi} and t_{Cj} is then given by:

$$P(t_{Oi}, t_{Cj}) = \frac{S(t_{Oi}, t_{Cj})}{N_S}, \tag{A1}$$

where $S(t_{Oi}, t_{Sj})$ is the number of simulated open-shut dwell-time pairs in the bin with mid–dwell times of t_{Oi} and t_{Cj}, and N_S is the total number of simulated dwell-time pairs in all the bins in the two-dimensional dwell-time distribution.

For a given kinetic scheme the probability $P[D(t_{Oi}, t_{Cj})]$ of making all the data observations in a single data bin with mid–dwell times of t_{Oi} and t_{Cj} is the probability of making the first observation *and* the second *and* the third . . . , which calculates as the product of all the individual probabilities, or

$$P[D(t_{Oi}, t_{Cj})] = [P(t_{Oi}, t_{Cj})]^{D(t_{Oi}, t_{Cj})}, \tag{A2}$$

where $P(t_{Oi}, t_{Cj})$ is given by Eq. A1.

It is easier to work with the logarithm of the likelihood so that the computation is with sums rather than products. The log-likelihood of making all the data observations in a single bin is thus denoted $L(t_{O1}, t_{Cj})$, and is given by

$$L(t_{Oi}, t_{Cj}) = \{\log [P(t_{Oi}, t_{Cj})]\} D(t_{Oi}, t_{Cj}), \tag{A3}$$

where log is a natural logarithm. Summing $L(t_{Oi}, t_{Cj})$ for all bins would then give $L(\theta)$, the log-likelihood for all the observations in the two-dimensional array of open-and-shut dwell times.

The above equations are stated in terms of the middwell-times of the bins. In practice, however, the actual dwell-times are of no consequence as long as the experimental and simulated two-dimensional dwell-time distributions are constructed in an identical manner. On this basis it is only necessary to make direct bin by bin comparisons between the simulated and experimental distributions. It follows from the above discussion that the log-likelihood $L(\theta)$ for all the individual data observations in a two-dimensional array with N_O rows and N_C columns is

$$L(\theta) = \sum_{i=1}^{N_O} \sum_{j=1}^{N_C} \{\log [S(i,j)/N_S]\} D(i,j), \tag{A4}$$

where N_O and N_C are the maximum number of bins required for the open and shut dwell-times, respectively, $D(i,j)$ is the number of data open-shut dwell-time pairs in bin (i,j), $S(i,j)$ is the number of simulated open-shut dwell-time pairs in bin (i,j), and N_S is the total number (in all bins) of the simulated open-shut dwell-time pairs.

One advantage of the iterative simulation method developed here is that it is not necessary to exclude any of the data from the fitting, and this includes the brief duration events. However, there may be instances where it would be necessary to restrict the fit to a limited range of dwell-times, such as after voltage steps of limited duration. Eqs. A5 and A6, which fit only those rows and columns with open and shut dwell-times within the specified time range, would then be used for the likelihood comparison.

$$L(\theta) = \sum_{i=a}^{b} \sum_{j=c}^{d} \{\log [S(i,j)/N_{RS})]\} D(i,j) \tag{A5}$$

$$N_{RS} = \sum_{i=a}^{b} \sum_{j=c}^{d} S(i,j), \tag{A6}$$

where a and b are the beginning and ending rows defining the open dwell times to be included in the fit, c and d are the beginning and ending columns defining the shut dwell times to be included in the fit, and N_{RS} is the (restricted) number of simulated open-shut dwell time pairs which fall within the range of open-and-shut dwell times to be included in the fit.

Eq. A4 or Eqs. A5 and A6 thus give the log-likelihood for the initial trial parameters θ (the initial estimates for the rate constants). An optimizing program is then used to search for the parameters θ which maximize the likelihood that the data are drawn from the distribution predicted by the given kinetic scheme. These values are the maximum likelihood estimates of the rate constants.

Because experimental and simulated data are treated identically, the fitting is performed without assumptions about missed events. However, in some instances it may be of interest, independent of the fitting, to estimate the fraction of events that are detected. This fraction is estimated from the ratio of the number of simulated events that are detected (as in Fig. 2 *C*) to the total number of simulated events (as in Fig. 2 *A*).

The maximum likelihood fitting procedure outlined above is presented in terms of open-shut dwell-time pairs, but the same equations could be used for maximum likelihood fitting of any binned data, providing that the experimental and simulated data are analyzed in an identical manner. With identical analysis and the incorporation of noise and filtering in the simulated data, any distortions in the experimental and simulated distributions arising from noise, filtering, missed events, binning, and sampling will be essentially identical for experimental and simulated data, and cancel out. The direct bin-by-bin comparison used in the likelihood calculation is very efficient, as the time required for the calculation is dictated by the relatively small number of bins, rather than the large numbers of simulated and experimental data points.

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