Estimation of single channel kinetic parameters from data subject to limited time resolution

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ABSTRACT The limited responsiveness of single-channel recording systems results in some brief events not being detected, and if this is ignored parameter estimation from the observed data will be biased. Statistical methods of correcting for this limited time resolution in a two-state Markov model have been proposed by Neher (1983. *J. Physiol.* (*Lond.*). 339:663–678) and by Colquhoun and Sigworth (1983. Single Channel Recording. 191–263). However, a numerical study by Blatz and Magleby (1986. *Biophys. J.* 49:967–980) indicated differences of 3–40% in the corrected values given by the two techniques. Here we explain why Neher's method produces biased results and the Colquhoun and Sigworth approach, which is no more difficult, provides reasonably accurate estimates.

INTRODUCTION

Understanding of membrane channel kinetics has been greatly advanced in recent years by the patch clamp technique and the complementary development of stochastic models of channel gating behavior. The models serve in part as a basis for statistical inference from single channel data. However, in systems where the kinetics are fast these data are degraded by the limited responsiveness of the recording system and filtering introduced to decrease noise. In such cases, it is preferable that the models take account of this limited time resolution to reduce bias in parameter estimation.

Correction methods have been discussed by several groups (Sachs and Auerbach, 1983; Colquhoun and Sigworth, 1983; Neher, 1983; Roux and Sauvé, 1985; Wilson and Brown, 1985; Blatz and Magleby, 1986; Ball and Sansom, 1987; Milne et al., 1988) The approach developed by Neher (1983) for a two-state Markov model was recently used by Prod'hom et al. (1987) in analyzing data arising from proton blockade of calcium channels. In an earlier study Blatz and Magleby (1986) showed that the Neher method could give parameter estimates substantially different from those obtained using the method due to Colquhoun and Sigworth (1983), the latter being more consistent with simulations. Here we examine the nature of these differences and the reliability of the two correction procedures.

THEORY AND NUMERICAL RESULTS

The following is based on a description of the channel kinetics as a continuous time equilibrium Markov process with two states, open and closed. Sojourn times less than a specified detection limit (dead-time) ξ are assumed to be missed (see Milne et al., 1988). For simplicity of presentation we assume, as did Neher (1983), that the detection limit is the same for both open times and closed times.

Let X and Y denote respectively typical (true) open times and closed times, having finite means μ_X and μ_Y, and let T and S denote the corresponding apparent quantities, the ξ-open time and ξ-closed time, having respective means μ_T and μ_S. In this notation Neher's approximate formulae, (B14 and B15), can be rewritten as

$$\mu_T = (\mu_X + \mu_Y)e^{\xi/\mu_Y} - \xi/(1 - e^{-\xi/\mu_Y}) \tag{1a}$$

$$\mu_S = (\mu_X + \mu_Y)e^{\xi/\mu_X} - \xi/(1 - e^{-\xi/\mu_X}), \tag{1b}$$

where for example μ_T and μ_S correspond respectively to Neher's $1/\alpha'$ and $1/\beta'$. The corresponding moment formulae (79 and 80) of Colquhoun and Sigworth (1983) are different from Eq. 1 being

$$\mu_T = (\mu_X + \mu_Y)e^{\xi/\mu_Y} - \mu_Y \tag{2a}$$

$$\mu_S = (\mu_X + \mu_Y)e^{\xi/\mu_X} - \mu_X. \tag{2b}$$

In either case, the method of estimation involves replacement of μ_T and μ_S by the corresponding sample means, $\bar{t}$ and $\bar{s}$, of the apparent quantities (obtained from a single channel record) and solution of the relevant pair of equations, Eqs. 1 or 2, to give estimates $\hat{\mu}_X$ and $\hat{\mu}_Y$ of the true mean open time and true mean closed time. Thus these are, in effect, both examples of moment estimation, in which estimating equations are obtained by equating expressions for suitable population moments to their sample values.

Neher recommended starting with $\mu_X = \bar{t}$ and $\mu_Y = \bar{s}$, to solve Eq. 1 iteratively. We have used Newton-Raphson

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iteration to find solutions for Eqs. 1 and 2 for each of five sets of values of $\bar{t}$, $\bar{s}$, and ξ given in the literature (Colquhoun and Sigworth, 1983; Blatz and Magleby, 1986; Prod'hom et al., 1987). The results are presented in Table 1, which shows also the percentage error in the solutions obtained using Eq. 1 relative to those using Eq. 2.

In general, it is not obvious whether the pair of Eqs. 1 has a solution, ($\hat{\mu}_X$, $\hat{\mu}_Y$), or whether such a solution is unique; the possibility of multiple solutions was not considered by Neher, yet for the CS parameter values (see Table 1), for example, a second solution is $\hat{\mu}_X$ = 0.1596, $\hat{\mu}_Y$ = 0.0917. For Eqs. 2, it has previously been observed in numerical examples considered by Colquhoun and Sigworth (1983) and Blatz and Magleby (1986) that there are usually two solutions. Mathematical analysis by Yeo et al. (1988) has confirmed that this is the case in general, and we would expect the same to hold for Eq. 1. For typical values as used in Table 1, one of the solutions, which we shall call the slow solution, has both its $\hat{\mu}_X$ and its $\hat{\mu}_Y$ value greater than the corresponding value for the other solution. Further comments regarding the two solutions and the choice of which is correct can be found in Colquhoun and Sigworth (1983) and Yeo et al. (1988).

The Eqs. 2, unlike Eqs. 1, are the exact moment equations based on the specified Markov model. Hence, the estimates $\hat{\mu}_X$ and $\hat{\mu}_Y$ derived using Eqs. 2 should be good statistically. In addition, it can be shown that they are the maximum likelihood estimates for the parameters μ_X and μ_Y under the approximate statistical model obtained by replacing the (nonexponential) densities of the adjusted apparent quantities, $U = T - \xi$ and $V = S - \xi$, by exponential densities with the correct means, as given by Eq. 2, a and b. (The means $\mu_U = \mu_T - \xi$ and $\mu_V = \mu_S - \xi$ are sometimes referred to as time constants in the approximate exponential distributions.) A more complex procedure, based on a better approximation using biexponential densities, generally yields estimates with less bias and greater precision (Yeo et al., 1988).

TABLE 1 Comparison of estimation methods

	Neher		Colquhoun and Sigworth	
Example	$\hat{\mu}_X$ (% error)	$\hat{\mu}_Y$ (% error)	$\hat{\mu}_X$	$\hat{\mu}_Y$
CS	1.1296 (28.55)	0.4056 (35.65)	0.8787	0.2990
BM_1	1.4537 (44.88)	0.1522 (51.74)	1.0034	0.1003
BM_2	1.1445 (14.48)	0.2496 (24.86)	0.9997	0.1999
P_1	0.9120 (5.47)	0.1366 (14.41)	0.8647	0.1194
P_2	0.3610 (7.70)	0.1461 (13.34)	0.3352	0.1289

Results for five literature examples using methods due to Neher (1983) and Colquhoun and Sigworth (1983). Colquhoun and Sigworth (1983), CS ($\bar{t}$ = 2.0, $\bar{s}$ = 0.6, ξ = 0.2); Blatz and Magleby (1986), BM_1 ($\bar{t}$ = 2.890, $\bar{s}$ = 0.216, ξ = 0.10), and BM_2 ($\bar{t}$ = 1.1778, $\bar{s}$ = 0.326, ξ = 0.10); Prod'hom et al. (1987), P_1 ($\bar{t}$ = 1.20, $\bar{s}$ = 0.16, ξ = 0.035), and P_2 ($\bar{t}$ = 0.48, $\bar{s}$ = 0.18, ξ = 0.035). (We used ξ = 0.035 in P_1 and P_2 because the bandwidth was 5 kHz). In each case, $\hat{\mu}_X$ and $\hat{\mu}_Y$ give respectively the slow solution estimates of μ_X and μ_Y obtained using Neher's method of correction (based on Eq. 1) and Colquhoun and Sigworth's method (based on Eq. 2). Percentage errors for Neher's estimates relative to the corresponding Colquhoun and Sigworth values are given in brackets. Estimates are in milliseconds and rounded to four decimal places.

By contrast in Neher's approach, the observed process, which is clearly non-Markov, is approximated by a two-state Markov process. This and the omission of some terms result in the incorrect moment expressions (Eq. 1). In effect, Neher's approximation can be viewed as resulting in single exponential approximations to the density functions of T and S, the ξ-open time and ξ-closed time (rather than the densities of the more relevant adjusted quantities, U and V). But these give incorrect (biased) approximations to μ_T and μ_S, which consequently result in biased estimates of μ_X and μ_Y.

To compare Neher's approximation with the exact moment expression, consider the difference between the right-hand sides (RHS) of Eqs. 2a and 1a:

$$\text{RHS (2a)} - \text{RHS (1a)} = \xi/(1 - e^{-\xi/\mu_Y}) - \mu_Y$$

$$= \frac{1}{2}\xi + \frac{1}{12}\xi^2/\mu_Y - \frac{1}{720}\xi^4/\mu_Y^3 + \text{higher order terms}, \quad (3)$$

where the resulting expression, which is valid for $\mu_Y > \xi$ (often the case), follows by expansion of $(1 - e^{-\xi/\mu_Y})^{-1}$ in powers of ξ. Observe that there are no terms in ξ^3 and that, because ξ and the coefficient of ξ^4 are small, the first two terms will generally give a good approximation to the error. Neglecting all terms other than the leading term, it is clear that an underestimate of $\xi/2$ would be made if Eq. 1a, rather than the exact expression Eq. 2a, were used to determine μ_T for specified μ_X and μ_Y. Analogous calculations based on Eqs. 1b and 2b lead to similar conclusions concerning determination of μ_S, though in cases where $\mu_X > \mu_Y$ consideration of the RHS shows that μ_S will be less in error than μ_T.

Of more practical interest are the respective errors a and b in $\hat{\mu}_X$ and $\hat{\mu}_Y$ obtained from Eq. 1, as opposed to Eq. 2, for given values, $\bar{t}$ and $\bar{s}$, of μ_T and μ_S. An error analysis is more difficult in this case, and it is necessary to resort to numerical examples or further approximations. The percentage (relative) errors for μ_X and μ_Y are generally larger than those for μ_T and μ_S, which can be explained as a result of a likelihood surface which is relatively flat in the neighborhood of the slow solution ($\hat{\mu}_X$, $\hat{\mu}_Y$) of Eq. 2. Suppose that $\mu_X \geq \mu_Y$. Expanding (2b) − (1b) gives

$$b \approx \tfrac{1}{2}\xi + \xi[a\mu_Y - (\mu_X - a)\xi/2]/[\mu_X(\mu_X + a)], \quad (4)$$

and a good approximation to the error b is $\xi/2$ whenever

μ_X is much greater than ξ. Substitution in Eq. 1a yields

$$a = [\mu_T + \xi(1 - e^{-\xi/(\mu_Y+b)})^{-1}]\, e^{-\xi/(\mu_Y+b)} - \mu_X - \mu_Y - b. \quad (5)$$

For the examples in Table 1 the value $\xi/2$ is close to the error in μ_Y in all cases. Using Eq. 5 with $b = \xi/2$, the percentage errors $100a/\mu_X$ are respectively 27.45, 43.64, 14.53, 5.53, 7.74, which are close to the actual values listed. If $\mu_Y > \mu_X$ then attention should be given first to the error in a by expanding (2a) − (1a).

The distributions of ξ-open times and ξ-closed times are known to be neither exponential nor biexponential, though often these provide adequate approximations (Yeo et al., 1988). Plots of the log density function, or of $\tilde{H}(x) = -ln[1 - \tilde{F}(x)]$ where $\tilde{F}(x)$ is the empirical distribution function (and in each case an exponential model is represented by a straight line readily appreciated by eye), as well as histograms and log histograms, can be used to assess the adequacy of fit for particular ion channel data sets (Blatz and Magleby, 1986; Sigworth and Sine, 1987; Yeo et al., 1988). A simulation of 5,000 ξ-open times and 5,000 ξ-closed times for the P_1 parameter values given in Table 1 ($\xi = 0.035$, $\mu_X = 0.8647$, $\mu_Y = 0.1194$) gave $\bar{t} = 1.20357$ and $\bar{s} = 0.16491$. In this particular case single exponential distributions provide good fits, apart from deviations near the origin (as plots of $\tilde{H}_U(u)$, $\tilde{H}_V(v)$ would indicate).

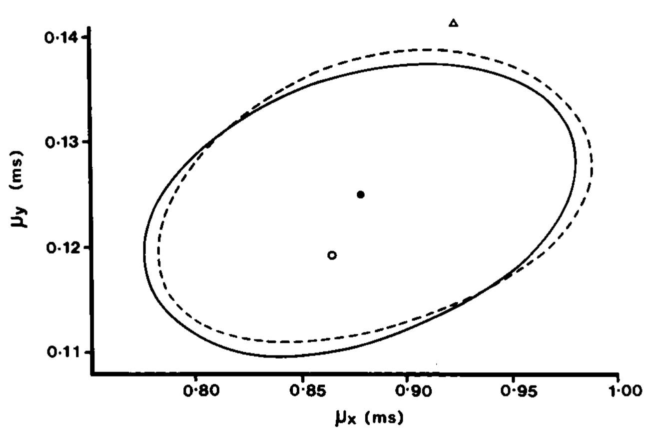

FIGURE 1 Simultaneous confidence regions for the parameters μ_X and μ_Y based on simulation of 5,000 ξ-open times and 5,000 ξ-closed times for the P_1 parameter values ($\xi = 0.035$, $\mu_X = 0.8647$, $\mu_Y = 0.1194$). Plots show 95% confidence regions for the slow solution, determined by using contours of the likelihood function based on a single exponential approximation (------), and by using the asymptotic bivariate normal distribution of the maximum likelihood estimators (——). The Neher estimates (0.9230, 0.1415), indicated by a triangle (Δ), lie outside the confidence region; the true value (*open circle*) and the Colquhoun and Sigworth estimates (*solid circle*) are shown for comparison. Note the difference in scale of the two axes, which underemphasizes correlation between parameter estimates.

Neher's method, as well as other moment methods, give estimates just of the mean values μ_X and μ_Y. If an exponential (or biexponential) distribution is used as an approximation to the distribution of ξ-open times (or ξ-closed times), then the method of maximum likelihood gives not only good estimates, but also information on their precision. For the above simulated data, in which $\hat{\mu}_X = 0.8774$ and $\hat{\mu}_Y = 0.1242$, Fig. 1 shows ~95% confidence regions for the slow solution (μ_X, μ_Y) (Yeo et al., 1988) obtained by two methods: using the contours of the likelihood function, and using the asymptotic bivariate normal distribution of the maximum likelihood estimators. The true parameter values (0.8647, 0.1194) lie well inside the regions, while the Neher estimates (0.9230, 0.1415) lie outside. This indicates that estimates using Eq.1 are not compatible with a null hypothesis specifying means as the true parameter values, providing further evidence of the inadequacy of this approach.

In summary, the Neher approach has been shown to be unsatisfactory relative to that of Colquhoun and Sigworth, which does provide a simple yet reasonably accurate estimation procedure for single channel kinetic parameters based on a two-state Markov model incorporating limited time resolution. If estimates of precision are required in addition to parameter estimates then likelihood methods can be used.

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