Poisson sampling-based inference for single ion channel data with time interval omission

F. G. BALL1, A. CHEN1 and M. S. P. SANSMO

1 Department of Mathematics, University of Nottingham, University Park, Nottingham, NG7 2RD, U.K.
2 Laboratory of Molecular Biophysics, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

SUMMARY
Patch-clamp recording allows investigations of the gating kinetics of single ion channels. Statistical analysis of kinetic data can enhance our understanding of channel gating at a molecular level. Experimental channel records suffer from time interval omission, i.e. failure to detect brief channel openings and closings. It is important to incorporate this phenomenon into statistical analyses of ion channel data. When time interval omission is ignored, the method of maximum likelihood can usually be used to estimate gating parameters from a single channel record. However, it is far more difficult to apply this method when time interval omission is incorporated. We present an alternative approach to parameter estimation based on Poisson sampling. A simulated homogeneous Poisson process is superimposed onto the channel record and inference is based on the numbers of points in successive open and closed sojourns, rather than on the sojourn times themselves. We describe the method for the two-state Markov model C<->O, although it is applicable to more general models. Computer-simulated data are used to demonstrate the efficacy of the method. Modifications of the method are discussed briefly.

1. INTRODUCTION
Patch-clamp recording (Sakmann & Neher 1983) allows direct measurement of the gating kinetics of single ion channels. Statistical analysis attempts to relate observed gating kinetics to underlying gating mechanisms. In particular, analysis of single-channel data allows construction of a model for the gating mechanism of an ion channel, and estimation of parameters for that model. Combining such analysis with site-directed mutagenesis studies will result in a molecular description of the channel gating mechanism.

Statistical inference is central to the development of channel gating models. Having selected, on the basis of analytical techniques, a plausible model which describes the gating behaviour of a particular channel, it is necessary to fit that model to the data, i.e. to obtain estimates of the model parameters. There has been some success in the use of maximum-likelihood methods to obtain parameter estimates from single-channel data (see, for example, Horn & Lange 1983; Horn & Vandenberg 1984; Chay 1989; Ball & Sansom 1989; Bates et al. 1989).

When analysing single-channel data, a complication arises. Very brief (typically less than ca. 100 ps) channel openings and closings are absent from experimental data (Colequhoun & Sigworth 1983). Failure to detect brief channel events results primarily from the finite response time of the amplifier, and is referred to as time interval omission. This may be modelled in terms of a fixed cut-off time. Events of duration less than the cut-off time are not observed. As has been discussed by several authors (see, for example, Roux & Sauvé 1985; Blatz & Magleby 1986; Milne et al. 1988; Ball & Sansom 1988; Hawkes et al. 1990, 1992; Crouzy & Sigworth 1990; Ball et al. 1992; Dabrowski & McDonald 1993), time interval omission has profound implications with respect to statistical analysis of channel data.

A procedure for modelling of time interval omission which may be applied to any single-channel gating mechanism has been developed by Ball et al. (1992). However, the problem of incorporating a complete treatment of time interval omission into inferential methods remains. Progress has been made in this area by Magleby & Weiss (1990), Fredkin & Rice (1992) and Hawkes et al. (1992). In an earlier study we investigated the problem in the context of a simple gating model and explored problems of model identifiability (Ball et al. 1990). In the current study we describe a Poisson sampling-based method which may be applied to any Markov gating model, including those with multiple open and closed states and with multiple pathways between these two sets of states. However, for simplicity of argument we illustrate this method by using a simple two-state model.

2. THEORY
We consider the following two-state Markov model of receptor-channel gating:

\[ C \overset{\lambda_1}{\rightleftharpoons} O. \]  \hspace{1cm} (1)

Thus successive open and closed sojourn times follow independent negative exponential random variables with mean \( \mu_o = \lambda_1^{-1} \) for open sojourns and mean \( \mu_c = \hspace{1cm} \)
\( \lambda_1 \) for closed sojourns. Time interval omission is modeled as failure to detect channel openings or closings of duration less than \( \tau \). Thus an observed open sojourn is defined as commencing with an opening of duration \( \geq \tau \), followed by a succession of openings of any duration which are separated by closings of duration \( < \tau \), and is immediately followed by a closing of duration \( \geq \tau \). An observed closed sojourn is defined similarly. Statistical interference for this model in the presence of time interval omission has been discussed by several authors including Goldqoom & Sigworth (1983), Blatz & Magleby (1986), Yeo et al. (1988), Milne et al. (1989), Ball et al. (1990), Clarke et al. (1992) and Ball & Davies (1992).

Let \( \theta = (\lambda_1, \lambda_2) \) and suppose that we wish to estimate \( \theta \) from observation of \( N \) successive pairs of observed open and closed sojourn times, \( t_1, s_1, t_2, s_2, \ldots, t_N, s_N \), say. The likelihood is given by

\[
L(\theta) = \prod_{k=1}^{N} \left( f_o(t_k) f_c(s_k) \right),
\]

where \( f_o(t) \) and \( f_c(s) \) are the probability density functions of typical observed open and closed sojourn times, respectively. Thus, in principle, \( \theta \) may be estimated by maximizing \( L(\theta) \). This is far from straightforward in practice owing to the lack of simple closed form expressions for \( f_o(t) \) and \( f_c(s) \). Closed form expressions for \( f_o(t) \) and \( f_c(s) \) were derived by Hawkes et al. (1990), but for computational purposes they are highly intensive and unstable for large \( t \) and \( s \). However, there are simple closed form expressions for the Laplace transforms of \( f_o(t) \) and \( f_c(s) \). Jalali & Hawkes (1992) used Tauberian arguments to obtain asymptotic approximations to \( f_o(t) \) and \( f_c(s) \), which are excellent even for relatively small \( t \) and \( s \), and could be used to approximate the likelihood (Hawkes et al. 1992). We shall explore an alternative approach to inference, Poisson sampling-based inference (see, for example, Basawa 1974), which makes explicit use of the above Laplace transforms.

Suppose that we superimpose onto the observed single-channel record a simulated Poisson process with known intensity, \( \nu \), say. For \( i = 1, 2, \ldots, N \), let \( m^0_i \) and \( m^1_i \) be the number of points of the simulated Poisson process that occur in the \( i \)th observed open and closed sojourns, respectively. Note that \( m^0_i, m^1_i, m^0_1, m^0_2, m^1_2, \ldots, m^0_N, m^1_N \) can be obtained by simulating independent Poisson random variables with means \( \nu t_1, \nu s_1, \nu t_2, \nu s_2, \ldots, \nu t_N, \nu s_N \). We shall base our inferences on the simulated Poisson counts \( m^0_1, m^1_1, m^0_2, m^0_2, m^1_2, m^0_3, m^1_3, \ldots, m^0_N, m^1_N \), rather than on the observed sojourn times \( t_1, s_1, t_2, s_2, \ldots, t_N, s_N \).

The reason for basing inferences on the simulated Poisson counts is that the likelihood can then be expressed in terms of appropriate derivatives of the Laplace transforms of \( f_o(t) \) and \( f_c(s) \) (see equation (3) below), thus circumventing the need to invert the Laplace transforms. Clearly, there is some loss of information in using the Poisson counts rather than the actual sojourn times. However, if we denote by \( X^{(i)}(t) \) the number of Poisson points occurring in a sojourn of length \( t \), then \( X^{(i)}(t) \sim \nu t \) for large \( \nu \), so the loss of information should not be too severe provided that \( \nu \) is sufficiently large. Moreover, as \( \nu \) tends to infinity, \( e^{-\nu t} X^{(i)}(t) \) converges to \( t \), so it is plausible that the Poisson sampling-based estimates of \( (\lambda_1, \lambda_2) \) will converge to the maximum-likelihood estimates from the actual sojourn times.

For \( k = 0, 1, \ldots \), let \( m^0_k \) be the number of observed open sojourns with \( m^0_0 = k \), and similarly define \( m^1_k \) \((k = 0, 1, \ldots)\). Let \( m^0_n = \max_{1 \leq i \leq N} m^0_i \) and \( m^1_n = \max_{1 \leq i \leq N} m^1_i \). The Poisson counts inherit mutual independence from the observed sojourn times. It follows that the likelihood is now given by

\[
L(\theta) = \prod_{k=0}^{m^0_n} \left( p^0_k \right)^{m^0_k} \prod_{k=0}^{m^1_n} \left( p^1_k \right)^{m^1_k},
\]

where \( p^0_k \) is the probability that a typical observed open sojourn contains \( k \) Poisson points \((k = 0, 1, \ldots)\) and \( p^1_k \) \((k = 0, 1, \ldots)\) are the corresponding probabilities for closed sojourns.

Now \( m^0_k \) follows a Poisson distribution with mean \( \nu t_k \), where \( t_k \) is an observation of a random variable with probability density function \( f_o(t) \). Thus

\[
p^0_k = \int_0^\infty \left( e^{-\nu t} \right)^k \frac{\nu t^k}{k!} f_o(t) dt
\]

is the Laplace transform of \( f_o(t) \), which is given by

\[
f_o(t) = \frac{\lambda_1 + \lambda_2}{\lambda^2 + (\lambda_1 + \lambda_2) \nu} e^{\lambda_1 t} e^{\lambda_2 t}.
\]

(see, for example, Milne et al. 1988), and \( \phi_0^{(k)}(v) \) is the \( k \)th derivative of \( \phi_0(v) \).}

In Appendix 1 we show that equations (3) and (4) imply that \( p^0_k \) \((k = 0, 1, \ldots)\) satisfy the recurrence relation

\[
\sum_{i=0}^{k} p^0_{k-i} q_i = \alpha_{k}, \quad (k = 0, 1, \ldots),
\]

where

\[
q_i = \begin{cases} 
\nu^2 e^{\nu (\lambda_1 + \lambda_2)} e^{\lambda_1 t_i} & \text{if } k = 0, 1, \ldots, \\
\nu e^{\nu (\lambda_1 + \lambda_2)} e^{\lambda_1 t_i} & \text{if } k = 0, 1, \ldots, \\
p_k & \text{if } k = 1, 2, \ldots, 
\end{cases}
\]

(6)

(7)

(8)

(9)

A similar recurrence relation for \( p^1_k \) \((k = 0, 1, \ldots)\) can be obtained by interchanging \( \lambda_1 \) and \( \lambda_2 \) in the above.

Note that equation (5) is straightforward to solve numerically. We first set \( k = 0 \) to obtain \( p^0_0 \), then \( k = 1 \) to obtain \( p^0_1 \), etc. Thus for given \( \theta = (\lambda_1, \lambda_2) \), the likelihood \( L(\theta) \), given by equation (2), can be
calculated. Hence $\theta$ can be estimated by maximizing $L(\theta)$ numerically using an iterative algorithm, such as the simplex method (Nelder & Mead 1965). As in all numerical maximization problems, several starting points should be used to guard against local maxima. Indeed, we expect $L(\theta)$ to have two local maxima as that is the case for inference based on approximating the likelihood surface had two peaks, broadly corresponding to the two solutions of the moment-estimating method of inference is working satisfactorily. The estimates of $(\mu_0, \mu_c)$ are always reasonably close to their known true values. Sometimes the method-of-moments estimate of $(\mu_0, \mu_c)$ is closer to the true value $(0.4, 10.0)$ and sometimes the Poisson sampling estimate $(0.0444, 0.1434)$, respectively. We then estimated the parameters $(\mu_0, \mu_c)$ from these observed sojourn times, using the method described in §2.

The means of observed open and closed sojourns, $\bar{\mu}_0$ and $\bar{\mu}_c$ say, can be derived from equation (4) and a similar expression for $\hat{f}(v)$, and are given by

$$\bar{\mu}_0 = (\lambda_1 + \lambda_2) e^{\lambda_2} - e^{\lambda_1}$$
$$\bar{\mu}_c = (\lambda_1 + \lambda_2) e^{\lambda_1} - e^{\lambda_1}$$

(8)

see, for example, Milne et al. (1988). Let

$$t = N^{-1} \sum_{i=1}^{N} t_i$$
$$s = N^{-1} \sum_{i=1}^{N} s_i$$

be the sample mean observed open and closed sojourn times, respectively. Then the method-of-moment estimator of $\theta = (\lambda_1, \lambda_2)$ is obtained by setting $$(\bar{\mu}_0, \bar{\mu}_c) = (t, s)$$ in equation (8) and solving for $(\lambda_1, \lambda_2)$. There will usually be two solutions for $(\lambda_1, \lambda_2)$, commonly known as the 'slow' and 'fast' solutions (see, for example, Colquhoun & Sigworth 1983) and we suggest using them as starting points for maximizing $L(\theta)$.

Error estimates for the parameters $\theta = (\lambda_1, \lambda_2)$ can be obtained via inversion of the information matrix (Cox & Hinkley 1975):

$$\text{cov}(\hat{\theta}) = \left[ H(\hat{\theta}) \right]^{-1}$$

(9)

where $\hat{\theta}$ is the estimate of $\theta$ that maximizes $L(\theta)$, $\text{cov}$ is the covariance matrix, and $H$ is the Hessian matrix of partial derivatives,

$$H_{ij} = \left[ \frac{\partial^2 \ln L}{\partial \lambda_i \partial \lambda_j} \right]_{\theta = \hat{\theta}}$$

(10)

An algorithm for calculating the Hessian matrix $H$ is given in Appendix 2.

In many studies, model (1) is parameterized by the mean sojourn times $(\mu_0, \mu_c)$ rather than by the rates $(\lambda_1, \lambda_2)$. Let $\theta = (\lambda_1, \lambda_2)$ be the maximum-likelihood estimate of $\theta$. Then by standard results for reparameterization of a model (see, for example, Cox & Hinkley 1974), the maximum-likelihood estimate, $\hat{\phi} = (\hat{\mu}_0, \hat{\mu}_c)$ say, of $\phi = (\mu_0, \mu_c)$ is given by $\hat{\phi} = (\lambda_1, \lambda_2)$, with corresponding information matrix

$$I(\hat{\phi}) = D(\hat{\phi}) I(\hat{\theta}) D(\hat{\phi})$$

where

$$D(\hat{\phi}) = \begin{bmatrix} \hat{\lambda}_1^2 & 0 \\ 0 & \hat{\lambda}_2^2 \end{bmatrix}$$

Finally, there is no reason why the rate $\nu$ of the superimposed Poisson process should be the same for open and closed sojourns. Indeed, often it will be appropriate to have different rates, $\nu_0$ and $\nu_c$ say, for open and closed sojourns as the lengths of observed open and closed sojourns can be markedly different. The Poisson counts $m_0, m'_0, m_1, m'_1, \ldots, m_N, m'_N$ are then obtained by simulating independent Poisson random variables with means $\nu_0 t_1, \nu_c s_1, \nu_0 t_2, \nu_c s_2, \ldots, \nu_0 t_N$, respectively, and the likelihood is modified accordingly. The problem of choosing $\nu_0$ and $\nu_c$ is discussed in §4.

3. RESULTS

We investigated the performance of Poisson sampling-based inference by considering model (1) with $(\lambda_1, \lambda_2) = (2.5, 0.1)$, so that $|\nu_0, \nu_c| = (0.4, 10.0)$. We simulated channel data from this model and applied time interval omission with a known detection limit $\tau$ to obtain a record of $N$ pairs of observed open and closed sojourn times. We then estimated the parameters $(\mu_0, \mu_c)$ from these observed sojourn times, using the method described in §2.

The means of observed open and closed sojourn times, $(\bar{\mu}_0, \bar{\mu}_c)$, can be calculated from equation (8). For the above, parameter values and experimentally reasonable values for $\tau$, $\bar{\mu}_0$ and $\bar{\mu}_c$ are quite different, e.g. when $\tau = 0.2$, $|\bar{\mu}_0, \bar{\mu}_c| = (0.6101, 16.7467)$. Thus we used distinct values for $\nu_0$ and $\nu_c$. The mean number of points in observed open and closed sojourns are the same if $\nu_0/\nu_c = \bar{\mu}_c/\bar{\mu}_0$. However, in any practical situation $\bar{\mu}_0$ and $\bar{\mu}_c$ are unknown, so we imposed the condition $\nu_0/\nu_c = 5/1$; recall that $t$ and $s$ are the sample means of observed open and closed sojourn times, respectively. We then chose $\nu_0$ and $\nu_c$ subject to this constraint so that $\nu_0 + \nu_c = \nu_{\text{TOT}}$, for different values of $\nu_{\text{TOT}}$. The problem of choosing $\nu_0$ and $\nu_c$ is discussed further in §4.

The results for $\nu_{\text{TOT}} = 10$ and various combinations of detection limit $\tau$ and record length $N$ are shown in table 1. For each combination of $N$ and $\tau$, $N$ observed open and closed sojourn times were simulated and the sample means $t$ and $s$ calculated. The parameters $(\mu_0, \mu_c)$ were then estimated by both the method-of-moments and by Poisson sampling. In each case the likelihood surface had two peaks, broadly corresponding to the two solutions of the moment-estimating equations. Table 1 just shows the 'true' estimates, i.e. those close to the known true values of the parameters. It is clear from table 1 that the Poisson sampling method of inference is working satisfactorily. The estimates of $(\mu_0, \mu_c)$ are always reasonably close to their known true values. Sometimes the method-of-moments estimate of $(\mu_0, \mu_c)$ is closer to the true value $(0.4, 10.0)$ and sometimes the Poisson sampling estimate. Note that, as one would expect, the covariance matrix $\text{cov}(\phi)$ for $\phi = (\mu_0, \mu_c)$ is decreasing with $N$ and increasing with $\tau$.

We next investigated the case $\tau = 0.2$ and $N = 5000$ more thoroughly. As before, we simulated 5000 observed open and closed sojourn times and calculated the sample means $t$ and $s$. They were $t = 0.6143$ and $s = 16.9002$. The corresponding slow and fast method-of-moments estimates of $(\mu_0, \mu_c)$ were $(0.4043, 10.1474)$ and $(0.0444, 0.1434)$, respectively. We then estimated $(\mu_0, \mu_c)$ by Poisson sampling with $\nu_{\text{TOT}} = 5, 10$ and 20, using the same set of simulated sojourns in each case. The results are shown in table 2. Note that the covariance matrix $\text{cov}(\phi)$ is decreasing with $\nu_{\text{TOT}}$, suggesting that the precision of the estimates of $(\mu_0, \mu_c)$ is increased by increasing the rate of the superimposed
Table 1. Estimates of $\mu_0$ and $\mu_c$ for various combinations of record length $N$ and detection limit $\tau$
(For each combination of $(N, \tau)$, the method-of-moments estimate $(\hat{\mu}_0^{(M)}, \hat{\mu}_c^{(M)})$, the Poisson sampling estimates $(\hat{\phi}_0, \hat{\phi}_c)$ and the covariance matrix $\text{cov} (\hat{\phi})$ of the Poisson sampling estimates are shown. The true parameter values were $(\mu_0, \mu_c) = (0.4, 10.0)$. For details see text.)

<table>
<thead>
<tr>
<th>$\tau$</th>
<th>$N = 1000$</th>
<th>$N = 5000$</th>
<th>$N = 10000$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>$(0.3863, 10.1718)$</td>
<td>$(0.4028, 9.9468)$</td>
<td>$(0.4063, 9.9181)$</td>
</tr>
<tr>
<td></td>
<td>$(0.3944, 10.0415)$</td>
<td>$(0.4009, 9.9054)$</td>
<td>$(0.4011, 9.9012)$</td>
</tr>
<tr>
<td></td>
<td>$\text{cov} (\hat{\phi})$</td>
<td>$\text{cov} (\hat{\phi})$</td>
<td>$\text{cov} (\hat{\phi})$</td>
</tr>
<tr>
<td></td>
<td>$2.0153 \times 10^{-3}$</td>
<td>$4.1489 \times 10^{-3}$</td>
<td>$2.0767 \times 10^{-3}$</td>
</tr>
<tr>
<td></td>
<td>$6.7808 \times 10^{-4}$</td>
<td>$1.3341 \times 10^{-3}$</td>
<td>$6.6669 \times 10^{-4}$</td>
</tr>
<tr>
<td>0.1</td>
<td>$(0.4105, 10.0190)$</td>
<td>$(0.4030, 9.8553)$</td>
<td>$(0.4015, 9.9152)$</td>
</tr>
<tr>
<td></td>
<td>$(0.4164, 9.9486)$</td>
<td>$(0.3999, 9.8436)$</td>
<td>$(0.3976, 9.8746)$</td>
</tr>
<tr>
<td></td>
<td>$2.2739 \times 10^{-3}$</td>
<td>$4.2399 \times 10^{-3}$</td>
<td>$2.1009 \times 10^{-3}$</td>
</tr>
<tr>
<td></td>
<td>$1.3674 \times 10^{-3}$</td>
<td>$2.7315 \times 10^{-3}$</td>
<td>$1.3718 \times 10^{-3}$</td>
</tr>
<tr>
<td>0.2</td>
<td>$(0.4059, 10.1647)$</td>
<td>$(0.4043, 10.1474)$</td>
<td>$(0.4020, 10.1352)$</td>
</tr>
<tr>
<td></td>
<td>$(0.4097, 10.2701)$</td>
<td>$(0.4026, 10.0794)$</td>
<td>$(0.3977, 9.9510)$</td>
</tr>
<tr>
<td></td>
<td>$2.3478 \times 10^{-4}$</td>
<td>$4.5609 \times 10^{-5}$</td>
<td>$2.2378 \times 10^{-5}$</td>
</tr>
<tr>
<td></td>
<td>$3.0189 \times 10^{-3}$</td>
<td>$5.9628 \times 10^{-4}$</td>
<td>$2.9588 \times 10^{-4}$</td>
</tr>
<tr>
<td>0.4</td>
<td>$(0.4036, 9.8491)$</td>
<td>$(0.3886, 9.8653)$</td>
<td>$(0.3996, 9.8661)$</td>
</tr>
<tr>
<td></td>
<td>$(0.4091, 9.9307)$</td>
<td>$(0.3903, 9.8990)$</td>
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</tr>
<tr>
<td></td>
<td>$2.8300 \times 10^{-4}$</td>
<td>$5.3111 \times 10^{-5}$</td>
<td>$2.6991 \times 10^{-5}$</td>
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<tr>
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<td>$1.4598 \times 10^{-3}$</td>
<td>$7.1667 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

Table 2. Poisson sampling estimates of $(\mu_0, \mu_c)$ for various values of $t_{\text{TOT}}$
(The true parameter values were $(\mu_0, \mu_c) = (0.4, 10.0)$.)

<table>
<thead>
<tr>
<th>$t_{\text{TOT}}$</th>
<th>slow solution</th>
<th>fast solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>$(0.4026, 10.1561)$</td>
<td>$(0.0445, 0.1446)$</td>
</tr>
<tr>
<td></td>
<td>$\text{cov} (\hat{\phi})$</td>
<td>$\text{cov} (\hat{\phi})$</td>
</tr>
<tr>
<td></td>
<td>$5.7542 \times 10^{-4}$</td>
<td>$3.7828 \times 10^{-2}$</td>
</tr>
<tr>
<td></td>
<td>$7.5542 \times 10^{-1}$</td>
<td>$2.4846 \times 10^{-1}$</td>
</tr>
<tr>
<td>10</td>
<td>$(0.4025, 10.1411)$</td>
<td>$(0.0445, 0.1445)$</td>
</tr>
<tr>
<td></td>
<td>$\text{cov} (\hat{\phi})$</td>
<td>$\text{cov} (\hat{\phi})$</td>
</tr>
<tr>
<td></td>
<td>$4.5608 \times 10^{-5}$</td>
<td>$3.2164 \times 10^{-2}$</td>
</tr>
<tr>
<td></td>
<td>$5.9982 \times 10^{-4}$</td>
<td>$2.0528 \times 10^{-2}$</td>
</tr>
<tr>
<td>20</td>
<td>$(0.4025, 10.1384)$</td>
<td>$(0.0445, 0.1443)$</td>
</tr>
<tr>
<td></td>
<td>$\text{cov} (\hat{\phi})$</td>
<td>$\text{cov} (\hat{\phi})$</td>
</tr>
<tr>
<td></td>
<td>$3.9703 \times 10^{-6}$</td>
<td>$5.2247 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>$5.2247 \times 10^{-4}$</td>
<td>$2.9353 \times 10^{-2}$</td>
</tr>
</tbody>
</table>

Poisson process. The likelihood surface for the case $t_{\text{TOT}} = 10$ is shown in figure 1. Note that there are two peaks, corresponding to the slow and fast solutions, with heights $ln L = -27.931.0$ and $ln L = -27.946.9$, respectively. Thus, as is well known for this two-state model, it is very difficult to distinguish between the 'true' and 'false' estimates of $(\mu_0, \mu_c)$ solely on the basis of likelihood. However, alternative methods can be used for deciding which is the true solution, such as using samples with different detection limits (Yeo et al. 1998), using samples with different agonist concentrations (Ball et al. 1990) and sample variance discrimination (Ball & Davies 1992).

Poisson sampling clearly introduces variation into the estimation of $(\mu_0, \mu_c)$, as for a given sequence of observed sojourn times the estimate $(\hat{\phi}_0, \hat{\phi}_c)$ depends on the simulated Poisson counts. We investigated this phenomenon by taking the observed sojourn times used to construct table 2 and figure 1, and doing 100 independent simulations of the Poisson counts (with $t_{\text{TOT}} = 10$) to obtain 100 estimates of $(\mu_0, \mu_c)$. For each simulation we only considered the estimate corresponding to the 'true' value of $(\mu_0, \mu_c)$. The sample mean and variance of the estimates of $\mu_0$ were now 0.3950 and 1.5444 $10^{-4}$, respectively. The corresponding results for $\mu_c$ were 9.9070 and 6.4747 $10^{-4}$. We next simulated 100 realizations of 5000 observed open and closed sojourn times, still with $\tau = 0.2$. For each realization we used a single Poisson sampling, with $t_{\text{TOT}} = 10$, to obtain an estimate of $(\mu_0, \mu_c)$. Thus we now obtained 100 independent estimates of $(\mu_0, \mu_c)$, each corresponding to the 'true' value. The sample mean and variance of the estimates of $\mu_0$ were now 0.4002 and 3.9838 $10^{-5}$, respectively, whereas those for the estimate of $\mu_c$ were 10.0076 and 2.8224 $10^{-2}$. Thus, for the above example, the percentage of the
Poisson-based inference for single-channel data

Figure 1. Contour plot of the log-likelihood for corresponding to the case \( t > T_{\text{TOT}} = 10 \) of table 2. The \( \beta_0 \) and \( \beta_c \) axes have been transformed to \( \beta_0 = \ln(\mu_0/0.4) \) and \( \beta_c = \ln(\mu_c/10.0) \). The heights of the contours are \(-38,500\) for the outermost contour, increasing in steps of \(17,500\) to \(-28,000\) for the two innermost contours.

Figure 1. Contour plot of the log-likelihood for \((\mu_0, \mu_c)\), corresponding to the case \( t_{\text{TOT}} = 10 \) of table 2. The \( \mu_0 \) and \( \mu_c \) axes have been transformed to \( \beta_0 = \ln(\mu_0/0.4) \) and \( \beta_c = \ln(\mu_c/10.0) \). The heights of the contours are \(-38,500\) for the outermost contour, increasing in steps of \(17,500\) to \(-28,000\) for the two innermost contours.

The total variance of the estimates owing to Poisson sampling is 38.76 for \( \mu_0 \) and 22.94 for \( \mu_c \). Although these percentages are quite large, they could be reduced either by increasing \( t_{\text{TOT}} \) or by taking the averages of the estimates of \( \mu_0 \) and \( \mu_c \) obtained from several independent realizations of Poisson sampling. Also, the variances are often quite small as long channel records are not uncommon.

4. DISCUSSION

We have presented Poisson sampling-based inference as a method of estimating single-channel kinetic parameters when the data is subject to time interval omission, and have described in detail its implementation for a two-state Markov model of receptor channel gating. The method has proved satisfactory for the two-state model. Furthermore, the method may be extended to multistate gating models, without any restrictions on the number of open states, the number of closed states, or the number of pathways connecting these two classes of state of the channel. Consequently, this procedure forms the basis of a general program for parameter estimation from single-channel kinetic data. The details of the general implementation of the method will be provided in a subsequent paper.

When using Poisson sampling-based inference, the choice of the intensities, \( v_0 \) and \( v_c \), of the superimposed Poisson process in observed open and closed sojourns rests with the experimenter. For ease of discussion, suppose initially that \( v_0 = v_c = v \), say. Now it is clear on intuitive grounds, and is borne out by the covariance matrices in table 2, that the precision of the parameter estimates is increasing with \( v \). The main difficulty in implementing the Poisson sampling method for the two-state model is calculating the count probabilities \( p_k^0 (k = 0, 1, \ldots) \) and \( p_k^c (l = 0, 1, \ldots) \). Clearly, the Poisson counts are increasing with \( v \), so choosing a larger value for \( v \) implies that \( p_k^0 \) and \( p_k^c \) will have to be calculated for larger values of \( k \). Now \( p_k^0 \) and \( p_k^c \) are calculated by solving recurrence relations, so the method can become both computationally too expensive and numerically unstable if too large a value for \( v \) is chosen.

Histograms of the Poisson counts for the example underlying figure 1 are shown in figure 2. Note that for both open and closed sojourns, large counts are comparatively rare. This suggests that it would be sensible to use censoring, i.e. fixing integers \( k_0 \) and \( k_c \) such that if an open Poisson count, \( m_0^0 \) say, is larger than \( k_0 \), only the fact that \( m_0^0 > k_0 \) and not the precise value of \( m_0^0 \) is used in constructing the likelihood \( L(\theta) \), and similarly for closed sojourns. The likelihood in equation (2) is then replaced by

\[
L(\theta) = \left( \prod_{k=0}^{k_0} (p_k^0)^{m_0^0} \right) \left( \prod_{l=0}^{k_c} (p_l^c)^{m_c^c} \right) \left( p_{k_0}^0 \right)^{n_{k_0}} \left( p_{k_c}^c \right)^{n_{k_c}},
\]

where \( \hat{p}_0^0 = 1 - \sum_{k=0}^{k_0} p_k^0 \) is the probability of there being more than \( k_0 \) Poisson points in an observed open sojourn, \( n_{k_0} \) is the number of observed open sojourns containing more than \( k_0 \) Poisson points, and \( \hat{p}_c^c \) and \( n_{k_c} \) are defined similarly but for closed sojourns. Thus if the above form of censoring is being used it is only necessary to calculate \( p_k^c \) for \( k = 0, 1, \ldots, k_0 \) and \( p_l^c \) for

\[
L(\theta) = \left( \prod_{k=0}^{k_0} (p_k^0)^{m_0^0} \right) \left( \prod_{l=0}^{k_c} (p_l^c)^{m_c^c} \right) \left( p_{k_0}^0 \right)^{n_{k_0}} \left( p_{k_c}^c \right)^{n_{k_c}},
\]

where \( \hat{p}_0^0 = 1 - \sum_{k=0}^{k_0} p_k^0 \) is the probability of there being more than \( k_0 \) Poisson points in an observed open sojourn, \( n_{k_0} \) is the number of observed open sojourns containing more than \( k_0 \) Poisson points, and \( \hat{p}_c^c \) and \( n_{k_c} \) are defined similarly but for closed sojourns. Thus if the above form of censoring is being used it is only necessary to calculate \( p_k^c \) for \( k = 0, 1, \ldots, k_0 \) and \( p_l^c \) for

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\]

where \( \hat{p}_0^0 = 1 - \sum_{k=0}^{k_0} p_k^0 \) is the probability of there being more than \( k_0 \) Poisson points in an observed open sojourn, \( n_{k_0} \) is the number of observed open sojourns containing more than \( k_0 \) Poisson points, and \( \hat{p}_c^c \) and \( n_{k_c} \) are defined similarly but for closed sojourns. Thus if the above form of censoring is being used it is only necessary to calculate \( p_k^c \) for \( k = 0, 1, \ldots, k_0 \) and \( p_l^c \) for

\[
L(\theta) = \left( \prod_{k=0}^{k_0} (p_k^0)^{m_0^0} \right) \left( \prod_{l=0}^{k_c} (p_l^c)^{m_c^c} \right) \left( p_{k_0}^0 \right)^{n_{k_0}} \left( p_{k_c}^c \right)^{n_{k_c}},
\]
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\[
\frac{v_T}{v_Q} \approx \frac{\mu_c}{\mu_o}, \quad \text{for } l = 0, 1, \ldots, k_c.
\]

The censoring levels \( k_0 \) and \( k_c \) should be as large as possible consistent with accurate and efficient computation of \( p_{00}^{(l)} \) and \( p_{l0}^{(l)} \). We suggest choosing the intensities \( v_o \) and \( v_c \) so that \( v_o/v_c = \mu_c/\mu_o \), for example by letting \( v_o/v_c = 1/s \), as in §3. Note that if censoring is being used it is no longer optimal to make \( v_T = v_o + v_c \) as large as possible, for if \( v_T \) is large then \( \mu_o v_Q + \mu_c v_T \) is the proportion of Poisson counts that are censored. It seems sensible to choose \( v_T = v_o + v_c \) as large as possible to ensure an accurate fit. We shall consider the optimal choice of \( v_T = v_o + v_c \) by using information criteria in a subsequent paper.

Finally, the choice of \( v_o \) and \( v_c \) may prove difficult for mechanisms having many states and sojourn time distributions with widely ranging time constants. A case in point is the closed time distribution for NMDA receptors with a low glutamate concentration, which may typically have up to five exponential components, with time constants ranging from \( 0.6 \text{ ms to } 600 \text{ ms} \) (Gibb & Colquhoun 1991). For such distributions, a high value for \( v_c \) would be desirable to cater for components with small time constants. But then sojourns corresponding to the large time constants might contain too many Poisson points for the method to be computationally feasible. However, in such circumstances it would be possible to censor the counts as described above, although further research is clearly required.

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APPENDIX 1. Derivation of recurrence relation (5) for \( p_k^0 \) \((k = 0, 1, \ldots)\).

Recall from (3) that
\[
\phi_k^0(v) = \left[1 - (v)^k / k!\right] \phi_k^0(v) \quad (k = 0, 1, \ldots). \tag{A1}
\]

Also, from (4),
\[
\phi_k^0(v) = h(v) / g(v), \tag{A2}
\]
where
\[
h(v) = (\alpha_1 + \lambda_2) v \tag{A3}
\]
and
\[
g(v) = \left[(v^2 + (\lambda_1 + \lambda_2) v) e^{(\alpha_1 + \lambda_2) v} + \lambda_1 \lambda_2. \tag{A4}\right]
\]

From (A2), \( \tilde{\phi}_0(v) g(v) = h(v) \), and hence by Leibniz's theorem
\[
\sum_{l=0}^{k} \binom{k}{l} \tilde{\phi}_k^{0-l}(v) g^{l}(v) = \tilde{h}(v) \quad (k = 0, 1, \ldots). \tag{A5}
\]

Multiplying both sides of (A5) by \( (-v)^k e^{-\lambda_2 r} / k! \) yields
\[
\sum_{l=0}^{k} \tilde{\beta}_{k-l} q_l = \alpha_k \quad (k = 0, 1, \ldots), \tag{A6}
\]
and
\[
\alpha_k = \left[(-v)^k / k!\right] \tilde{h}(v) \quad (k = 0, 1, \ldots). \tag{A7}
\]

Let
\[
g_k(v) = \left[(v^2 + (\lambda_1 + \lambda_2) v) e^{(\alpha_1 + \lambda_2) v}\right] \quad (k = 0, 1, \ldots), \tag{A8}
\]
and
\[
g_k(v) = e^{(\alpha_1 + \lambda_2) v}. \tag{A9}
\]

Then, again using Leibniz's theorem,
\[
g_k^{(k)}(v) = \sum_{l=0}^{k} \binom{k}{l} \tilde{g}_l^{(k-l)}(v) g_2^{l}(v) \quad (k = 1, 2, \ldots). \tag{A10}
\]

Now \( g_k^{(k)}(v) = 0 \) \((l = 2, 3, \ldots)\), and it is easily verified that
\[
g_k^{(k)}(v) = e^{(\alpha_1 + \lambda_2) v} \left[(v^2 + (\lambda_1 + \lambda_2) v + (2v + \lambda_1 + \lambda_2) \times k \tau^{-1} + \kappa(\kappa - 1) \tau^{-2}\right] \quad (k = 1, 2, \ldots). \tag{A11}
\]
Hence,
\[
g_k = \left[(-v)^k / k!\right] e^{(\alpha_1 + \lambda_2) v} \left[(v^2 + (\lambda_1 + \lambda_2) v + (2v + \lambda_1 + \lambda_2) \times k \tau^{-1} + \kappa(\kappa - 1) \tau^{-2}\right] \quad (k = 1, 2, \ldots). \tag{A12}
\]

and (6) follows. The case \( k = 0 \) follows directly from (A4) and (A6). Finally, (7) follows immediately from (A3) and (A7).

APPENDIX 2. Calculation of information matrix

In this Appendix we derive an algorithm for calculating the Hessian matrix \( H \) given by (10). From (2),
\[
\ln L = \sum_{k=0}^{m_0} p_k^0 \ln p_k^0 + \sum_{l=0}^{m_1} \phi_l^0 \ln \phi_l^0,
\]
so
\[
\frac{\partial^2 \ln L}{\partial \lambda_i \partial \lambda_j} = \sum_{k=0}^{m_0} \left[1 - \frac{\partial^2 p_k^0}{\partial \lambda_i \partial \lambda_j} \right] + \sum_{l=0}^{m_1} \left[1 - \frac{\partial^2 \phi_l^0}{\partial \lambda_i \partial \lambda_j} \right] \quad (i, j = 1, 2, \ldots). \tag{A13}
\]

Define the differential operators \( D \) and \( D^{(2)} \) by
\[
D = \begin{bmatrix} \frac{\partial}{\partial \lambda_1} & \frac{\partial}{\partial \lambda_2} \\ \frac{\partial}{\partial \lambda_1} & \frac{\partial}{\partial \lambda_2} \end{bmatrix} \quad \text{and} \quad D^{(2)} = \begin{bmatrix} \frac{\partial^2}{\partial \lambda_1^2} & \frac{\partial^2}{\partial \lambda_1 \lambda_2} & \frac{\partial^2}{\partial \lambda_2 \lambda_1} & \frac{\partial^2}{\partial \lambda_2^2} \\ \frac{\partial^2}{\partial \lambda_1 \lambda_2} & \frac{\partial^2}{\partial \lambda_1^2} & \frac{\partial^2}{\partial \lambda_1 \lambda_2} & \frac{\partial^2}{\partial \lambda_2^2} \end{bmatrix},
\]
where \( T \) denotes transpose.

We shall describe a method for calculating \( D p_k^0 \) and \( D^{(2)} p_k^0 (k = 0, 1, \ldots) \). A similar method can be used for calculating \( D q_k^0 \) and \( D^{(2)} q_k^0 (k = 0, 1, \ldots) \), and then \( H \) can be calculated from (A8).

Differentiating (5) we obtain
\[
\sum_{l=0}^{k} \tilde{\beta}_{k-l} q_l = \alpha_k \tag{A9}
\]
where, differentiating (6),
\[
D q_k = \left[\right]. \tag{A10}
\]
and, differentiating (7),
\[
D q_k = \left[\right]. \tag{A11}
\]

The recurrence relation (A9) determines \( D p_k^0 (k = 0, 1, \ldots) \) recursively.

To calculate \( D^{(2)} p_k^0 (k = 0, 1, \ldots) \), first note that, for functions \( f \) and \( g \),
\[
D^2 (fg) = f D^2 g + g D^2 f + (D f) (D g)^T + (D g) (D f)^T. \tag{A12}
\]

Thus, applying \( D^{(2)} \) to both sides of (5), we obtain
\[
\sum_{l=0}^{k} \left[\right] + \left[\right] = D^{(2)} \alpha_k \tag{A13}
\]
where, by appropriate differentiation of (6),
\[
D^{(2)} q_k = \left[\right]. \tag{A14}
\]
and, by appropriate differentiation of (7),
\[
D^{(2)} \alpha_k = \left[\right]. \tag{A15}
\]

Again, the recurrence relation (A10) can be solved recursively to yield \( D^{(2)} p_k^0 (k = 0, 1, \ldots) \).