# Estimating the number of channels in patch recordings

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ABSTRACT The estimation of the number of channels in a patch was assumed to be equivalent to the estimation of the binomial parameter $n$. Seven estimators were evaluated, using data sets simulated for a range of parameters appropriate for single channel recording experiments. No single estimator was best for all parameters; a combination of estimators is a possible option to avoid the biases of individual estimators. All estimators were highly accurate in estimating $n$ in the case that $n = 1$. For $n \leq 4$ the simplest estimator, the maximum number of simultaneously open channels, was the best, For larger values of $n$ the best estimators were Bayesian.

## INTRODUCTION

Single channel recording from membrane patches is a hit or miss proposition. The number of channels in a patch depends on many factors, including the size of the patch, the density of channels, and the distribution of channels in the membrane (i.e., random vs. "patchy"). In some cases it may be possible to control the expected number of channels in each patch. For example, the size of the patch pipette may be altered, or the density of channels may be manipulated by controlling the expression of the channels. Also, for patches with an excessive number of channels, the probability of opening may be reduced (e.g., by control of membrane potential or the concentration of an agonist or blocker) to prevent overlapping open events. For many types of analysis of gating kinetics, however, it is important to know the number of channels in a patch. This paper addresses the estimation of channel number.

The presently used estimators of channel number rely on two assumptions, that the channels in a patch are (*a*) homogeneous (i.e., each channel has the same gating properties), and (*b*) independent (i.e., the gating behavior of a channel does not affect its neighbors). Under these assumptions the number of open channels in a patch will obey a binomial distribution, namely

$$b(k|n,p) = (n!/[k!(n-k)!])p^k(1-p)^{n-k}, \quad k = 0, 1, \ldots, n, \quad (1)$$

where the probability of exactly $k$ simultaneously open channels is $b(k|n,p)$, $n$ is the number of channels in the patch, and $p$ is the probability for an individual channel to be open. Thus the estimation of channel number is equivalent to the estimation of the parameter $n$ in Eq. 1. For repeated samples, i.e., trials, $n$ and $p$ are assumed to be constant.

Three methods are commonly used for estimating $n$ in electrophysiological experiments. The simplest is to observe the maximum number of simultaneously open channels, $k_{max}$, over a long period of continuous recording, or for many trials when channels are repeatedly activated by voltage or an agonist. The MAX estimate of $n$ is $k_{max}$. The second method is a Method of Moments Estimate (MME). For the binomial distribution the mean, $\mu = np$, and the variance, $\sigma^2 = np(1-p)$. Rearranging shows that $n = \mu^2/(\mu - \sigma^2)$. The sample mean, $\bar{x}$, and sample variance, $s^2$, are used to estimate $\mu$ and $\sigma^2$, respectively, in this equation. Each experiment consists of $m$ independent trials, with $k_i$ $(i = 1, \ldots, m)$ simultaneously open channels in each trial. The sample mean, $\bar{x}$, is $\bar{x} = \Sigma_{i=1}^{m} k_i/m$. The sample variance $s^2 = \Sigma_{i=1}^{m}(k_i - \bar{x})^2/m$. The third method is the Maximum Likelihood Estimate (MLE) of $n$. The MLE must be evaluated numerically for this problem (e.g., see Patlak and Horn, 1982).

All three of the aforementioned estimators are problematic. MAX is biased, since $k_{max}$ is always less than or equal to $n$. Both MME and MLE have shortcomings when $p$ is small, i.e., when $\mu \cong \sigma^2$. If, in a given sample, $s^2$ happens to be greater than $\bar{x}$, then the MME of $n$ is negative, and the MLE is infinite (Olkin et al., 1981). Also, when $\bar{x} \cong s^2$, both MME and MLE are highly unstable for small variations in the data (Olkin et al., 1981; Casella, 1986). Examples of unstable data sets have been reported in experimental literature (e.g., see Patlak and Horn, 1982). In the past few years stabilized versions of MME and MLE, and other estimators of $n$, have been introduced. Some of these estimators will be evaluated, specifically in the context of the type of data encountered in single channel experiments.


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## THEORY AND METHODS

## Evaluation of estimators

The general approach to evaluation of the estimators of $n$ was the following. Data sets containing $m$ trials were simulated under the assumption that each $k_i$ was an independent sample from a binomial population with known $n$ and $p$. The values of $n$, $p$, and $m$ were chosen from ranges of values in patch clamp experiments. For small values of $n$, i.e. $<50$, each $k_i$ was simulated by adding the results of a sum of $n$ Bernoulli trials, each with probability $p$ of success. The outcome of each trial was determined by comparing $p$ with a uniformly distributed, double precision, random deviate, generated by the FORTRAN function RAN1 (p. 196 in Press et al., 1986). For $n \geq 50$ a double-precision acceptance-rejection method was used for each $k_i$ (p. 208 in Press et al., 1986).

One hundred data sets were simulated for each triplet $(n, p, m)$, and each estimator was evaluated by its relative error, namely the square root of the average value of $(\hat{n} - n)^2/n^2$ for all 100 data sets, where $\hat{n}$ is the nearest integer of the estimate of $n$. Also the fraction of correct estimates (i.e., the fraction of data sets in which $\hat{n} = n$) was tabulated.

## Choice of parameters $n$, $p$, and $m$ for simulations

Patches typically come in two flavors, either patches with a handful of channels (i.e. $<5$), or patches with tens to hundreds of channels. In the former case the exact number of channels in a patch may be critical in interpretation of the data. In the latter case the behavior of single channels is observable by making the open probability, $p$, reasonably low, for example by using a low concentration of an agonist. With the above considerations simulations used values of $n$ ranging from 1 to 256, in powers of 2. The parameter $p$ may range from 0 to 1.0 in patch clamp experiments, and was allowed a range from 0.01 to 0.9 in these simulations, with the provision that the mean number of openings be moderate ($np \leq 3$), as in usual experiments.

The number of independent samples, $m$, in patch clamp experiments typically varies from $\sim 100$ to several thousand. (The significance of the term "independent" in such experiments will be explored in the Discussion, below). I used values of 50, 100, 500, 1,000, 2,000, and 5,000 for these simulations. Data sets were not generated when the total number of open events was expected to be $<6$, i.e., when $mnp < 6$. A total of 219 $(m, n, p)$ triplets satisfied the above criteria and, as stated above, 100 data sets were simulated for each of these triplets.

## $f_{max}$ criterion

A criterion is introduced here that depends on the fraction, $f_{max}$, of the data set in which $k_i = k_{max}$. As $f_{max}$ approaches 1, the probability that $k_{max} = n$ approaches 1 (see Appendix).

To determine a critical value of $f_{max}$ appropriate for this study, data were simulated over the entire range of values of $(n, p, m)$. For each triplet 100 data sets were simulated; the number of data sets in which $k_{max} = n$ is plotted against the mean value of $f_{max}$ in Fig. 1 *A*. This figure shows that $k_{max} = n$ with high probability when $f_{max} \geq 0.1$. Fig. 1 *B* shows the distribution of $f_{max}$ for all simulated data sets in which $n > k_{max}$. As $f_{max}$ increases, the probability that $n > k_{max}$ decreases to $<0.02$ when $f_{max} \geq 0.1$. An $f_{max}$ criterion will be used in two of the estimators described below.

## Seven estimators

Seven estimators of $n$ were considered. Most of the estimators yield noninteger values, but in all cases the nearest integer was taken as the

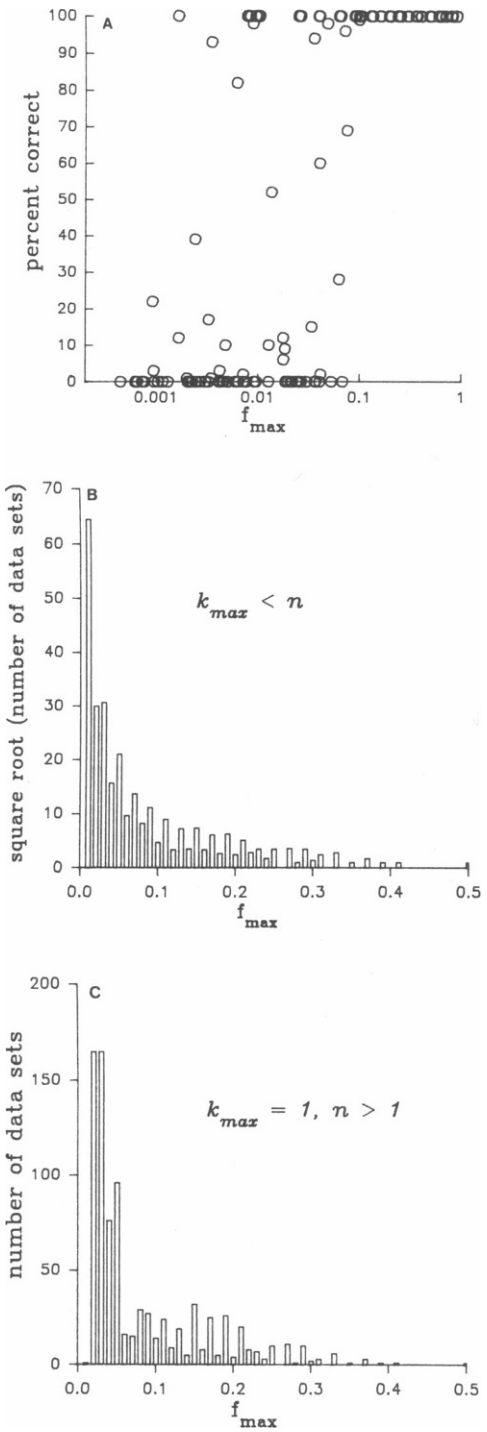


FIGURE 1 Evaluation of $f_{max}$. 100 data sets were simulated over the entire range of $(n, p, m)$. *A* Plots the number of data sets in each triplet in which $k_{max} = n$ against $f_{max}$. *B* plots the distribution of $f_{max}$ for all data sets in which $k_{max} < n$. *C* plots the distribution of $f_{max}$ for all data sets in which $k_{max} = 1$ and $n > 1$.

estimate. I have not included MLE, because it can produce infinite estimates in unstable cases, and is comparable to MME in stable cases (Olkin et al., 1981).

Estimator 1: MAX. MAX takes $k_{max}$ in a sample as the estimate of $n$. MAX is stable but biased. From Eq. 1 we see that $b(n|n, p) = p^n$, suggesting that MAX will tend to be a good estimator when $p$ is large and when $n$ is small. The number of trials, $m$, is large (on the order of ~1,000) in patch recording experiments. This also increases the probability that $k_{max} = n$, which is

$$1 - (1 - p^n)^m.$$

Furthermore, if $f_{max}$, the proportion of independent trials in which $k_i = k_{max}$, is large enough (see above), then MAX is a good estimator of $n$.

Estimator 2: JK. A jackknife estimator (JK) of $n$ eliminates bias in MAX of the order $1/m$ (Olkin et al., 1981). It is defined as

$$JK = k_{max} + [(m - 1)/m][k_{max} - k_{[m-1]}],$$

where $k_{[m-1]}$ is the $[m - 1]$th ordered value of $k_i$, namely the largest value of $k_i$ remaining after removing a single observation $k_{max}$ from the data set. Note that $k_{[m-1]}$ may equal $k_{max}$.

Estimator 3: MME. MME, discussed above, is $(\bar{x})^2/(\bar{x} - s^2)$.

Estimator 4: GC(α, β). GC(α, β) belongs to a class of estimators known as Bayesian. Such estimators postulate "prior densities" for the parameters being estimated. For example, the parameter $p$ in the binomial distribution ranges between 0 and 1, and we can imagine that, in a given experiment, $p$ is selected at random from a prior density with a range between 0 and 1. We might even have a reasonable idea what value $p$ has before estimating it, based on prior information or assumptions. The appropriate prior density function for a parameter is called the conjugate prior (Ferguson, 1967, chapter 1). Bayesian estimators have a variety of virtues, including stability for small samples (see below).

GC(α, β) uses the Beta(α, β) distribution as the conjugate prior for $p$ and the Gamma(α + β, δ) distribution as the conjugate prior for $n$. The Beta distribution has a range between 0 and 1, and the Gamma distribution between 0 and ∞. The mean of Beta(α, β) is $\alpha/(\alpha + \beta)$, and its variance is $\alpha\beta/(\alpha + \beta)^2(\alpha + \beta + 1)$. The mean of Gamma(α + β, δ) is $(\alpha + \beta)\,\delta$, and its variance is $(\alpha + \beta)\delta^2$. These are joint prior distributions, and use the posterior mean of $n$ as its estimate. The values of α and β must be assumed, but the parameter δ is derived from the data set. The choice of α and β is a prior assumption about the binomial parameter $p$. The chosen values reflect both an initial guess for the numerical value of $p$, given by the mean of the Beta distribution, and a reflection of the confidence of this initial guess, given by the variance of the Beta distribution (see below). Details are found in Günel and Chilko (1989), where it is shown that GC(α, β) is stable and in some cases superior to other estimators. Briefly,

$$GC(\alpha, \beta) = \frac{\int_0^\infty G_1(y)e^{-y}\,dy}{\int_0^\infty G_2(y)e^{-y}\,dy}, \qquad (2)$$

where

$$G_1(y) = g(y)[t(y)]^{\alpha+\beta}$$
$$G_2(y) = G_1(y)/t(y)$$
$$t(y) = y\delta + k_{max}$$
$$\delta = \bar{x}/\alpha$$

and

$$g(y) = \frac{\prod_{i=1}^{m}\prod_{j=0}^{k_i-1}[t(y) - j]}{\prod_{j=1}^{\alpha+\Sigma k_i}[u(y) - j]}$$

for integer values of α and β, and where $u(y) = (\alpha + \beta) + mt(y)$. Eq. 2 is solved by using the Laguerre-Gauss approximation,

$$\int_0^\infty G(y)e^{-y}\,dy \cong \sum_{i=1}^{\infty} w_i G(y_i), \qquad (3)$$

where $y_i$ and $w_i$ are tabulated in Abramowitz and Stegun (1970, p. 923). The same integer estimates were obtained for 8 and 10 terms of Eq. 3 for selected examples; therefore 8 terms were used for the more extensive Monte Carlo simulations. Logarithms were used to prevent overflow in the numerical evaluation of $g(y)$. In the evaluation of this estimator α and β were, except where indicated, both set at 1, equivalent to a uniform prior for $p$, with a mean value of 0.5.

For Bayesian estimators, such as GC(α, β), the assumed parameters of the prior distributions are especially important for small samples, in this study for a small number of trials, $m$. For larger samples the prior information becomes dominated by the data, because each sample leads to an updating of the prior parameters (Ferguson, 1967, chapter 1). Fig. 2 shows the behavior of GC(α, β) as a function of α, β, and $m$. Data were simulated for $p = 0.1$ and $n = 6$, with 100 data sets generated for each value of $m$. Fig. 2*A* shows the mean value of GC(α, β) as a function of $m$. MME is also shown for comparison. All estimators converge to the correct answer as $m$ increases. As might be expected, the estimator that is consistently closest to $n = 6$ is GC(1, 9), because it assumes a prior for $p$ with a mean of 0.1. However, higher or lower values of this "initial guess" for $p$ affect the estimates only for a smaller number of trials. Fig. 2 *B* shows the relative error for the same estimators and the same data sets. Fig. 2 also demonstrates the striking instability of MME for small samples. It should be noted that the prior confidence in a particular initial guess for $p$ can be augmented by increasing $\alpha + \beta$ while keeping $\alpha/(\alpha + \beta)$ constant. For integer values the most ignorant choice is $\alpha = \beta = 1$.

Estimator 5: MMES. MMES is a stabilized version of MME that chooses between two estimators, depending on the ratio $\bar{x}/s^2$, which is a measure of the stability of the sample (Olkin et al., 1981). MME is used for stable data sets.

Specifically, MMES = Max $(s^2\phi^2/(\phi - 1), k_{max})$, where

$$\phi = \begin{cases} \bar{x}/s^2 \\ \text{Max}\{(k_{max} - \bar{x})/s^2,\ 1 + 1/\sqrt{2}\} \end{cases}$$

$$\text{if } \bar{x} \geq (1 + 1/\sqrt{2})s^2 \quad \text{(stable)}$$
$$\text{if } \bar{x} < (1 + 1/\sqrt{2})s^2 \text{ (unstable)}$$

Olkin et al. (1981) show that MMES is considerably more stable than either MME or MLE when $\bar{x}$ is close to $s^2$.

Estimator 6: MIX1. MIX1 and MIX2 combine some of the above estimators in an attempt to improve their accuracy. MIX1 employs the estimator GC(α, β) and the criterion $f_{max}$. Since MME performs well for so-called stable data sets, I used the stability criterion of Olkin et al. (1981) for choosing between MME and GC(1, 1). Thus MIX1 is defined by the following sequential strategy.

If $f_{max} \geq 0.1$, MIX1 = $k_{max}$.

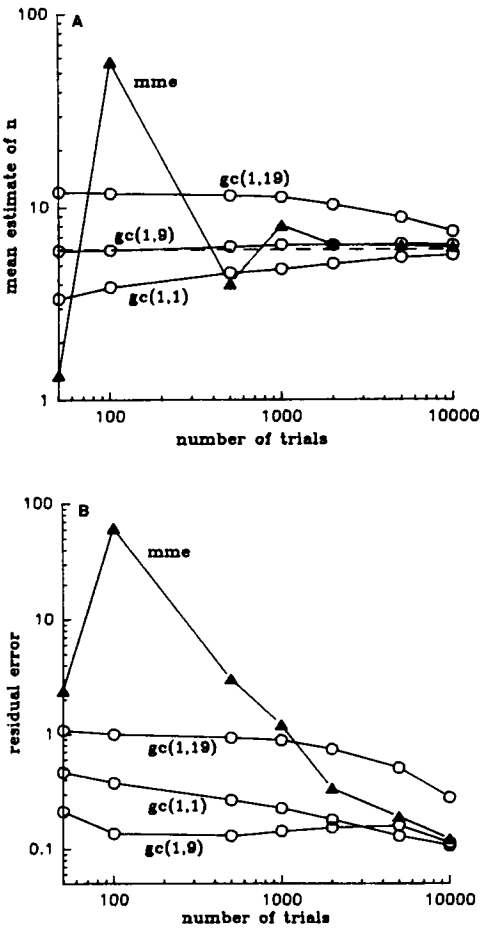


FIGURE 2 Effect of number of trials, $m$, on GC($\alpha$, $\beta$) and MME. 100 data sets were simulated for each $m$, with $n$ and $p$ set at 6 and 0.1. *A* plots the mean estimate of $n$ for each estimator. *B* plots the relative error for the same estimators and data sets.

If $f_{max} < 0.1$, then

$$\text{MIX1} = \begin{cases} \text{MME} & \text{if } \bar{x} \geq (1 + 1/\sqrt{2})s^2 \text{ (stable)} \\ \text{GC(1, 1)} & \text{if } \bar{x} < (1 + 1/\sqrt{2})s^2 \text{ (unstable)} \end{cases}.$$

Estimator 7: MIX2. MIX2 combines the $f_{max}$ criterion with MMES. Thus, If $f_{max} \geq 0.1$, MIX2 = $k_{max}$. Else, MIX2 = MMES.

## RESULTS

All seven estimators were perfect in estimating $n$ when the value used for the simulations was $n = 1$, meaning that for the entire range of $p$ and $m$, every data set produced the estimate 1 for each estimator. Table 1 shows the relative error for the estimates of $n$ and the fraction of correct estimates for different ranges of $n$. The ranking of estimators, based on relative error, for all data sets was (best-to-worst): MIX1 > GC(1, 1) > MIX2 > MAX > JK > MMES ≫ MME. For $n$ small (i.e. ≤4) the ranking was MAX > MIX2 > MMES > JK > MIX1 > GC(1, 1) ≫ MME. For $n > 4$ the ranking was GC(1, 1) > MIX1 > JK > MMES > MIX2 > MAX ≫ MME. These lists show that no estimator dominates, in terms of relative error, but clearly point out the inadequacy of MME, as noted by previous authors. The rankings also show that, if $n \leq 4$, then the simplest estimator, MAX, was the best. MAX was correct in estimating $n$ in 83% of the data sets when $n$ was between 2 and 4.

The choice of estimators also depends on the range of $p$ used in the simulations (Table 2). For $p$ between 0.01 and 0.4 the ranking (best to worst) was MIX1 > GC(1, 1) > MMES > MIX2 > JK > MAX ≫ MME, and between 0.5 and 0.9 was MAX > JK > MIX2 > MIX1 > MMES > MME > GC(1, 1). For $n$ between 2 and 4 *and* $p$ between 0.01 and 0.4 the ranking was

TABLE 1 **Relative error, $(\hat{n} - n)^2/n^2$, for the estimates of $n$ and the fraction of correct estimates ($f_{cor}$) for three ranges of $n$**

| | MAX | JK | MME | GC(1, 1) | MMES | MIX1 | MIX2 |
|---|---|---|---|---|---|---|---|
| $n$ = 2 to 256 (163 triplets): | | | | | | | |
| Error | 0.307 | 0.307 | 2.54 | 0.293 | 0.308 | 0.289 | 0.305 |
| $f_{cor}$ | 0.535 | 0.522 | 0.518 | 0.534 | 0.534 | 0.548 | 0.535 |
| $n$ = 2 to 4 (104 triplets): | | | | | | | |
| Error | 0.083 | 0.093 | 1.31 | 0.107 | 0.091 | 0.100 | 0.086 |
| $f_{cor}$ | 0.833 | 0.803 | 0.754 | 0.790 | 0.826 | 0.812 | 0.828 |
| $n > 4$ (59 triplets): | | | | | | | |
| Error | 0.703 | 0.686 | 4.71 | 0.620 | 0.690 | 0.622 | 0.690 |
| $f_{cor}$ | 0.008 | 0.026 | 0.100 | 0.082 | 0.018 | 0.082 | 0.018 |

100 data sets were simulated and analyzed for each triplet ($n, p, m$).

TABLE 2 **Relative error and the fraction of correct estimates ($f_{cor}$) for two ranges of $p$**

| | MAX | JK | MME | GC(1, 1) | MMES | MIX1 | MIX2 |
|---|---|---|---|---|---|---|---|
| $p$ = 0.01 to 0.4 (115 triplets): | | | | | | | |
| Error | 0.435 | 0.435 | 3.59 | 0.405 | 0.429 | 0.405 | 0.429 |
| $f_{cor}$ | 0.341 | 0.324 | 0.330 | 0.366 | 0.346 | 0.368 | 0.345 |
| $p$ = 0.5 to 0.9 (48 triplets): | | | | | | | |
| Error | 0.001 | 0.003 | 0.023 | 0.024 | 0.018 | 0.012 | 0.006 |
| $f_{cor}$ | 0.999 | 0.995 | 0.968 | 0.936 | 0.982 | 0.978 | 0.992 |
| $p$ 0.01 to 0.4 *and* $n$ = 2 to 4 (56 triplets): | | | | | | | |
| Error | 0.153 | 0.170 | 2.41 | 0.179 | 0.154 | 0.175 | 0.154 |
| $f_{cor}$ | 0.691 | 0.638 | 0.572 | 0.666 | 0.692 | 0.670 | 0.688 |

100 data sets were simulated for each triplet ($n, p, m$). Only cases where $n > 1$ are compiled here.

MAX > MMES > MIX2 > JK > MIX1 > GC(1, 1) ≫ MME. As might be expected, all estimators improved as $p$ increased.[1]

## Does $n = 1$?

Although all estimators behaved perfectly when $n = 1$, they all erred occasionally by choosing $\hat{n} = 1$ when, in fact, $n > 1$. The fraction of data sets in which $n$ was incorrectly estimated as 1 was defined as $p_{err1}$ and ranged between 0.051 and 0.053 for all estimators for all triplets $(n, p, m)$, except MME, where it was 0.063. This error was lower for high values of $p$. If $p \geq 0.1$, for example, $p_{err1}$ was between 0.0098 and 0.012 for all estimators, except MME, where it was 0.021. For $p \geq 0.2$, the errors reduced to the level of ~0.002. This error was also reduced by increasing the number of independent observations, $m$, although the effect of increasing $m$ was less dramatic than that of increasing $p$. For example, a tenfold increase in $m$ from 500 to 5,000 reduced $p_{err1}$ by a factor of ~2.6. Fig. 1 *C* also shows that $p_{err1}$ decreases as $f_{max}$ increases for all data sets in which $k_{max} = 1$. For $f_{max} > 0.13$, $p_{err1} < 0.02$. The estimator MAX was always among those with the lowest $p_{err1}$, indicating that all estimators tend to estimate $n$ as 1, unless $k_{max} > 1$. In practice, therefore, the best strategy to reduce $p_{err1}$ is to try experimentally to maximize $p$ and look for overlapping events, using as many trials as possible. In addition, if the fraction of trials with 1, rather than 0, openings is greater than 0.13 (Fig. 1 *C*), then the number of channels is likely to be 1.

## Is $n$ small or large?

The methods used for analysis of single channel data may depend on whether patches have a small or large number of channels. In the former case the exact number is used to correct the data; in the latter other methods can be applied, although it is generally desirable to know whether the number is large or small (eg. Jackson, 1985). For precision, I define $p_{errs}$ as the probability of believing $n$ is small, i.e., $\leq 4$, when it is actually $> 4$; conversely $p_{errb}$ is the probability of incorrectly estimating $n$ as big, i.e. $> 4$.

In terms of $p_{errs}$, the estimators were ranked (best to worst): MME > GC(1, 1) > MIX1 > JK > MMES > MIX2 > MAX (Table 3). As above, this error can be reduced by increasing $p$. For $p \geq 0.1$ the values of $p_{errs}$ are reduced by a factor of ~1.7. For $p \geq 0.2$ the estimator with the lowest $p_{errs}$ (0.0042) was GC(1, 1). A tenfold increase in number of independent trials, $m$, reduced $p_{errs}$ by a factor of ~1.3.

TABLE 3 **Errors for incorrectly estimating $n$ as small or large**

| | MAX | JK | MME | GC(1, 1) | MMES | MIX1 | MIX2 |
|---|---|---|---|---|---|---|---|
| $p_{errs}$ | 0.475 | 0.426 | 0.148 | 0.330 | 0.446 | 0.335 | 0.448 |
| $p_{errb}$ | 0.000 | 0.019 | 0.056 | 0.047 | 0.006 | 0.028 | 0.004 |

$p_{errs}$ is the probability of incorrectly estimating $n$ as small, i.e. $\leq 4$, when it is actually $> 4$; conversely $p_{errb}$ is the probability of incorrectly estimating $n$ as big, i.e. $> 4$.

The probability of incorrectly estimating $n$ as $> 4$, $p_{errb}$, tended to be smaller than $p_{errs}$. The estimators in this case were ranked: MAX > MIX2 > MMES > JK > MIX1 > GC(1, 1) > MME (Table 3). Clearly MAX was incapable of overestimating $n$.

The above considerations show that underestimation is more difficult to avoid than overestimation for these seven estimators, i.e., $p_{errs}$ tends to be larger than $p_{errb}$. When $p_{errs}$ is considered to be the more serious error, MME and GC(1, 1) are the least likely estimators to lead to the wrong conclusion. However, as discussed above, neither may provide the most accurate estimates for $n$.

## DISCUSSION

The estimation of the binomial parameter $n$ has a long history of problems (see Discussion in Olkin et al., 1981), and no single estimator is best under all circumstances. The difficulties usually arise when $p$ is small and $n$ is large, and in the extreme the binomial distribution approaches a Poisson distribution with a single parameter $\lambda = np$. The purpose of this paper is the evaluation of several commonly used, and a few novel, estimators with data simulated within the range of $(n, p, m)$ encountered in single channel experiments. Even with this narrow focus, no single estimator was the optimal choice for all situations. However, it is relatively simple to employ a combination of estimators for commonly encountered problems. Furthermore, the application of some prior information greatly improves the choices among estimators, and in some cases, the accuracy of the estimators, as discussed below. All estimators perform better when $p$ is larger. Therefore, a confident knowledge of $n$ is improved by maximizing $p$ as much as experimentally possible.

The methods in this paper may be used to address three common questions: (*a*) does a patch with $k_{max} = 1$ have one or more than one, channels?, (*b*) does a patch have few or many channels?, and (*c*) if a patch has only a few channels, how many are there? The answers to these questions are summarized here.

[1]Some caution is required in the interpretation of Tables 1 and 2 because of the restricted range of $(n, p, m)$. This is due to the limitation that $np \leq 3$. Thus, higher values of $n$ were always associated with lower values of $p$.

In the absence of prior assumptions the best method to determine if a patch has more than one channel is to try to maximize $p$ experimentally, and look for overlapping openings, using as many trials as possible. Unfortunately the confidence in this procedure is difficult to quantify. It would be useful, for example, to know how many trials are necessary to feel confident that, if $k_{max} = 1$, then $n = 1$. However, the probability that $k_{max} > 1$ depends both on $n$ and $p$ (Eq. 1), neither of which are known in general. Confidence in the decision that $n = 1$ is increased, however for large values of $f_{max}$, the fraction of independent samples in which $k_i = 1$ (Fig. 1 *C*). When $n > 1$ the maximum value of $b(1|n,p)$, estimated by $f_{max}$, is 0.5 (Appendix 1). This value is obtained when $n = 2$ and $p = 0.5$. Therefore, values of $f_{max} > 0.5$, when $k_{max} = 1$, indicate that $n = 1$. A less extreme critical value of $f_{max}$, leading to errors on the order of 2%, is 0.13 (Fig. 1 *C*). An alternative approach to this problem is given by Colquhoun and Hawkes (1990), who show that a measurement of $f_{max}$ and open time may be used to estimate the probability that the patch has two, rather than one, channels.

The decision whether a patch has a small ($\leq 4$) or large number of channels has complementary errors. The error of incorrectly estimating $n$ as $>4$ was lowest for MAX and MIX2. The error of incorrectly estimating $n$ as $\leq 4$ was lowest for MME and GC(1, 1).

For a patch with a few (i.e. $\leq 4$) channels the best estimator of channel number was MAX. Even if $p$ is also small (i.e. $\leq 0.4$), MAX was the best estimator of $n$ for patches with $\leq 4$ channels (Table 2).

The least compute-intensive strategy for estimation of $n$ is to use MAX and MME to determine whether $n$ is large or small. If $n$ is small MAX is the best estimator.

## Use of prior information

Some of the estimators in this study make use of prior assumptions about the data. Two types of prior assumptions were employed. First, a Bayesian estimator, GC($\alpha$, $\beta$), requires an assumption about the prior distributions of $p$ and $n$. Second, simulation was used to determine critical values of $f_{max}$.

The Bayesian estimator, GC(1, 1), and MIX1 (which is not itself Bayesian, but uses GC[1, 1]), were the best estimators for $n > 4$. Bayesian estimators have the appealing property that the prior parameters are updated for every data sample such that, in the limit, the data dominate the prior assumptions (Fig. 2). This property is combined with high stability for a low number of trials. I used $\alpha = \beta = 1$ as an ignorant prior in this study. Perhaps the most intriguing possibility here is the judicious use of more precise assumptions. For example, the probability $p$ is often known reasonably accurately to the experimentalist, who obtains many recordings from the same biological preparation under similar conditions. This information can be incorporated into the choices for $\alpha$ and $\beta$.

The use of $f_{max}$ is a way to assess the precision of $k_{max}$ as an estimator of $n$. Because useful critical values were not evident, they were determined by simulation. The critical values in this study were obtained for a rather large range of $(n, p, m)$, and could be made more precise by defining appropriate ranges for experimentally encountered parameters.

## Real life considerations

This study is limited to an evaluation of estimators for the binomial parameter $n$, with parameters for simulations chosen from a range commonly encountered in single channel recording. Besides the assumption that each $k_i$ is an independent sample from a binomial distribution, no provisions are made for the accuracy of the determination of $k_i$. These points will be considered briefly here.

In several experimental studies the number of open channels was well fit by a binomial distribution (e.g., see Patlak and Horn, 1982; Blatz and Magleby, 1986). The binomial distribution for the number of open channels assumes that channels are identical and gate independently of one another, and that $n$ and $p$ are constants. Failure to fit a binomial distribution using a goodness-of-fit criterion, would indicate that one of these assumptions is incorrect (Glasbey and Martin, 1988; Dabrowski et al., 1990). However, this may not be a powerful test (Yéramian et al., 1986; Dabrowski et al., 1990; Dabrowski and McDonald, 1991), and the possibility remains that subtle interactions among neighboring channels in a patch, or that microheterogeneity, either in the channels or in the membrane, may invalidate the generality of the binomial distribution.

Obtaining independent samples is not necesssarily simple. Two types of experiments are commonly used, either repeated applications of stimuli, such as voltage steps, or continuous recording under stationary conditions. In the former type, each stimulus is assumed to be an independent trial, and an isochronal measurement of the number of open channels is possible (e.g., Patlak and Horn, 1982). In the latter type, independent samples could be obtained by counting the number of open channels at regularly spaced intervals, as long as these intervals are longer than the relaxation time for the gating kinetics of the channels. This relaxation time can be estimated from the autocovariance of the patch current during a continuous recording (Liebovitch and Fischbarg, 1985). For the MAX estimator, however, one should observe the entire experiment and look for the maximum number of open channels. An alternative approach to estimation of $n$ also disregards the relax-

ation time of gating and uses, instead, the relative proportion of time spend at each conductance level as the representation of the binomial distribution (Dabrowski et al., 1990). The stability of their method has not, however, been evaluated in some of the extreme cases explored in this paper.

Ion channels have been reported to show a slow switching between modes of gating (e.g., see Hess et al., 1984; Horn et al., 1984), implying that $p$ may not be constant. This is a problem of stationarity, which can be tested, for example, using the methods in Dabrowski and McDonald (1991). Nonstationarity could lead to an underestimation of the number of channels, if recordings are made, fortuitously or deliberately, during a period of high activity (Colquhoun and Hawkes, 1990).

A final sobering consideration is the ability to measure accurately the number of open channels at a chosen time. This ability depends on several factors, including the signal-to-noise ratio for the open channel current over the baseline current, and the kinetics of gating. Rapid gating can lead to inaccurate measurements, due to the effects of filtering and sampling rate (Colquhoun and Sigworth, 1983). These problems are exacerbated in situations in which the number of overlapping openings is large.

## APPENDIX

If $f_{max}$, which estimates $b(k_{max}|n,p)$, is greater than a constant $<1.0$, then $n = k_{max}$. This is shown by the following lemma.

*Lemma.* If $n > k_{max}$, then $\sup_{n,p} b(k_{max}|n,p) = b(k_{max}|k_{max}+1, k_{max}/[k_{max}+1]) \leq 0.5$.

Inspection of the binomial equation (Eq. 1) shows that, if $n = k_{max}$, the maximum value of $b(k_{max}|n,p) = 1.0$. If $n > k_{max}$, a supremum can be found by taking logarithms of Eq. 1 and differentiating with respect to $p$. Thus,

$$\frac{\partial \ln b(k_{max}|n,p)}{\partial p} = k_{max}/p - (n - k_{max})/(1-p).$$

Setting equal to zero and solving for $p$ gives

$$p = k_{max}/n,$$

which, for $n > k_{max}$, is maximum when $n = k_{max} + 1$. Finally, $b(k_{max}|k_{max}+1, k_{max}/[k_{max}+1])$ is a decreasing function of $k_{max}$, and equals 0.5 for $k_{max} = 1$.

Thus, $b(k_{max}|k_{max}+1, k_{max}/[k_{max}+1])$ may be used as a critical value. If $f_{max}$ exceeds this value, then $k_{max} = n$. This idea was tested by simulation over the entire range of $(n,p,m)$ and, although never in error, it was also found not to be useful for estimation of $n$, because $f_{max}$ exceeded the critical value only when $p$ was so large that all estimators correctly determined $n$.

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