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A χ^2 Test for Model Determination and Sublevel Detection in Ion Channel Analysis

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Abstract. A χ^2 test is proposed that provides a means of discriminating between different Markov models used for the description of a measured (patch clamp) time series. It is based on a test statistic constructed from the measured and the predicted number of transitions between the current levels. With a certain probability, this test statistic is below a threshold if the model with a reduced number of degrees of freedom is compatible with the data. A second criterion is provided by the dependence of the test statistic on the number of data points. For data generated by the alternative model it increases linearly. The applicability of this test for verifying and rejecting models is illustrated by means of time series generated by two distinct channels with different conductances and by time series generated by one channel with two conductance levels. For noisy data, a noise correction is proposed, which eliminates noise-induced false jumps that would interfere with the test. It is shown that the test can also be extended to aggregated Markov models.

Key words: (aggregated) Markov model — Model discrimination — Multi-channel records — Noise — Patch clamp — Subconductance level

Introduction

Ion channels in biological membranes switch spontaneously or under the influence of drugs or messengers between conductive and non-conductive states. The kinetics of this so-called gating can be described by Markov models (Korn & Horn, 1988; Colquhoun & Hawkes, 1995). The adequate Markov model is not *a priori* known, and it is a serious challenge to reveal this model from the measured time series of the channel current, especially if more than one channel contributes to that current.

Model identification is done on different levels of complexity. The determination of the number of channels in a measured record can be tested by using a simple binomial distribution (Neher, Sakmann & Steinbach, 1978; Horn, 1991; Draber, Schultze & Hansen, 1993). For a comparison of several different methods *see* Horn (1991). Rydén (1995) suggested maximum split data estimates for the determination of the number of states in a hidden Markov model (Remark: We do not include aggregated Markov models in the class of hidden Markov models in order to distinguish between the two models). Empirical methods for model identification based on the Akaike information criterion and the Schwarz criterion are given by Ball & Sansom (1989). Horn & Vandenberg (1984), Blatz & Magleby (1986) and Horn (1987) considered tests for model discrimination on the basis of dwell times (Akaike information criterion and likelihood ratio test). A very time-consuming and complex approach for model discrimination is the Markov chain Monte Carlo method (Ball et al., 1999; Hodgson & Green, 1999; Schouten, 2000; De Gunst, Künsch & Schouten, 2000). Less complicated is the 2-dimensional dwell-time analysis (Blatz & Magleby, 1989; Song & Magleby, 1992; Colquhoun, Hawkes & Srodzinski, 1996). However, this latter approach requires a large amount of data. The likelihood ratio test for aggregated Markov models was applied by Horn & Lange (1983) and by Wagner & Timmer (2000). Fredkin & Rice (1992a) proposed a likelihood ratio test for hidden Markov models. Sansom et al. (1989) used the empirical Schwarz criterion for the discrimination of Markov, fractal, diffusion and re-

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Abbreviations: SLM, sublevel model; 2CM, two-channel model; C, C′, closed state; O, open state; S, sublevel state; SNR, signal-to-noise ratio.

lated models for the channel gating mechanism. (The Markov model performed best.)

However, often the experimenter is interested in a simple, but mathematically solid test for accepting or rejecting suggested models. In the case of independent, identically distributed (iid) random variables and in the case of Markov processes, there exist two standard test procedures: the likelihood ratio test (LRT) and the χ^2 test (besides other, e.g., the Wald test). Both tests have been applied for model identification in Markov processes (likelihood ratio test: evolution: Carroll & Corneli, 1995; Yang & Roberts, 1995; clinical trials: Patel & Khatri, 1981; sequential modulation: Yu & Kuo, 1996; χ^2 test: weather data: Sundararaj & Ramachandra, 1975; Singh & Sutradhar, 1989; sedimentary sequences: Sharp & Markham, 2000; both: language recognition and cryptography: Ganesan & Sherman, 1993, 1994). These two tests are asymptotically equivalent (for iid random variables *see*, e.g., Lehmann, 1986; Stuart & Ord, 1991; for Markov chains *see* Billingsley, 1961; Basawa & Rao, 1980). Hence for a sufficiently large number of observation points there is no difference in the behavior of the tests. For a finite number of data points the tests differ. For a comparison *see*, e.g., Singh & Sutradhar, 1989; Ganesan & Sherman, 1994 and the discussion and references in Stuart & Ord, 1991. As expected, each test has its advantages depending in a complicated way on the data, but on the whole there seems to be very little difference in most situations.

Here a χ^2 test is provided for models with a reduced number of degrees of freedom, i.e., a reduced number of independent variables sufficient to describe the observed kinetics (with respect to a general model). Because of the similarity of the tests even for a finite number of observations, it is to be expected that the results for the χ^2 test for Markov models should apply to the likelihood-ratio test for Markov models.

There is a considerable number of systems to which the χ^2 test can be applied. An important class consists of ensembles of channels with two different current levels (Patlak, 1988; Schild, Ravindran & Moczydlowski, 1991; Tyerman, Terry & Findley, 1992; Draber & Schultze, 1994; Root & MacKinnon, 1994). Here, two different scenarios may apply: Two independent channels with different conductivities each (two-channel model, 2CM), or one channel with two different conductive states (sublevel model, SLM). Draber and Schultze (1994) suggested an empirical method to distinguish between 2CM and SLM. Below, a mathematical test is presented, which can reject or admit the 2CM by virtue of its reduced number of degrees of freedom. This χ^2 test is especially simple to apply because only the number of transitions of the time series is needed. No high computational power as in the case of aggregated or especially hidden Markov models is required.

The application of the proposed χ^2 test is demonstrated by simulations, which particularly examine the admissibility of the theoretical asymptotic statements. A simple 2CM compared with an SLM demonstrates the behaviour of the test statistic and its ability to distinguish between similar models. Then, the application of the test is extended to multichannel records and to noisy data (including a method to correct the effect of noise in high-noise records). Even though the test is based on the assumption of Markov models, simulations show that it can also be used for aggregated Markov models.

Theory

The χ^2 test for Markov chains is a means of testing whether a set of experimental data can be explained by a Markov model which has a reduced number of degrees of freedom compared to the general model. Originally, a χ^2 test applies to independent, identically distributed random variables. However, it has been shown that this test can also be extended to Markov chains with a finite number of states although the measured data are not independent and not identically distributed (Billingsley, 1961; Basawa & Rao, 1980; Caliebe, 1996).

We consider a time-homogeneous Markov chain of m states, for simplicity numbered from 1 to m. Let $P = (p_{i,j})_{i,j\in\{1,\ldots,m\}}$ be the related transition matrix and π the initial distribution. For a temporal sequence $x = (x_0, x_1, \ldots, x_n)$ of $n + 1$ data points the likelihood (i.e., probability) of x is given by

$$L(x,P) = \pi(x_0)\prod_{k=1}^{n} p_{x_{k-1},x_k} = \pi(x_0)\prod_{i,j=1}^{m} p_{i,j}^{n_{ij}} \qquad (1a,b)$$

In the medium term, the product is taken over the probabilities p_{x_{k-1},x_k} that the time series takes the value x_k at sampling point k given the value x_{k-1} at sampling point $k - 1$. In the last term, the product is taken over the probabilities $p_{i,j}$ of transitions from state i to state j of the Markov chain by combining equal factors, $n_{ij} = n_{ij}(x)$ is the number of transitions from state i to state j observed in the data x. In Eq. 1*b* the information contained in 2-D dwell-time histograms (Song & Magleby, 1992) is lost. This is relevant for aggregated Markov models, but not for Markov models that are considered in the following test.

Remark: Consider a Markov process Y in continuous time (e.g., the standard model for an ion channel). Such a process is characterized by the initial distribution π and the matrix of rate constants $K = (k_{i,j})_{i,j\,\in\,\{1,..,m\}}$. If we observe Y at times $n \cdot \Delta t$ (Δt is the fixed sampling interval) then the process X_n: =

$Y_{n \cdot \Delta t}$ is a discrete-time Markov chain with transition matrix (Bharucha-Reid, 1960)

$$P = \exp(\mathrm{K}\Delta\mathrm{t}) = \sum_{\mathrm{n}=0}^{\infty} \frac{1}{\mathrm{n}!}(\mathrm{K}\Delta\mathrm{t})^{\mathrm{n}}. \tag{2}$$

For ion-channel data it is a common procedure to regard the sampled observations as a time-discrete Markov chain, thereby approximating the underlying continuous process (Albertsen & Hansen, 1994; Becker et al., 1994; Colquhoun & Hawkes, 1995; Fredkin & Rice, 1992*a*, *b*, 2001).

The initial distribution π in Eq. 1 is fixed and is not considered as a variable for the statistical model. The probability distribution of the Markov chain is completely determined by the transition matrix P. This stochastic matrix P ($p_{i,j} \geq 0$, $\Sigma_j\ p_{i,j} = 1$) is described by at most $m^2 - m$ parameters. Our aim is to decide whether the transition matrix P belongs to a certain subset H of transition matrices. H is called the hypothesis, and H^C, the set of all transition matrices not belonging to H, is called the alternative. We are interested in subsets H which can be parameterized by fewer parameters than $m^2 - m$, i.e.,

$$H = \{P = P(\phi) \in \mathbb{R}^{m \times m} : \phi \in \Phi\}$$

with $\Phi \subset \mathbb{R}^s$, $s < m^2 - m$: Every transition matrix $P \in H$ can be identified as a vector of parameters $\phi \in \Phi$. The actual function $P(\phi) = (p_{i,j}(\phi))_{i,j \in \{1,..,m\}}$ is determined by the hypothesis (i.e., the model to be tested).

In the case of a patch-clamp record with two different conductivities, the hypothesis H is the model of two independent channels or, more precisely, all corresponding transition matrices P of the macro-channel (i.e., the Markov model consisting of all states of the ensemble of the two channels (Colquhoun & Hawkes, 1977, 1990; Blunck et al., 1998)). The $p_{i,j}(\phi)$ are the transition probabilities of the macro-channel, whereas the components of ϕ, ϕ_l, $l = 1, 2, ..., s$, are the transition probabilities of the individual channels.

$\hat{\phi}$, the value of $\phi \in \Phi$ such that the data x has the highest probability, is determined by

$$\hat{\phi} = \hat{\phi}(x) = \underset{\phi \in \Phi}{\operatorname{argmax}}\ L(x, P(\phi)). \tag{3}$$

To simplify notation we assume that $\hat{\phi}$ is unique, if existent. Calculating $\hat{\phi}$ is usually done by solving the likelihood equations

$$\frac{\partial L}{\partial \phi_l}(x, P(\phi)) = 0, \qquad l = 1, 2, \ldots, s, \tag{4}$$

in ϕ. This solution is in many cases unique and equal to $\hat{\phi}$. For the following we assume that a solution of Eq. 4, if existent, is unique and equals $\hat{\phi}$. The $\hat{\phi}_l$ are used for the numerical calculation of $p_{i,j}(\hat{\phi})$ which are employed in the suggested χ^2 test for H: It is based on the test statistic

$$T_{th} := T^n_{th}(x) := \sum_{\substack{i,j=1 \\ p_{i,j}(\hat{\phi})>0}}^{m} \frac{[n_{ij} - n_i p_{i,j}(\hat{\phi})]^2}{n_i p_{i,j}(\hat{\phi})} \tag{5}$$

with $n_i = n_i(x) = \sum_{j=1}^m n_{ij}$ being the number of data points at which state i is measured. (Recall that n_{ij} is the number of transitions from state i to state j. Note that transitions from state i to i, n_{ii}, are included in the sum for calculating n_i. For each $n \in \mathbb{N}$ we have the equality $n = \sum_{i=1}^m n_i$.) Later on the test statistic T_{th} will be substituted by $T = T_{ap}$ in order to take into account the supposed underlying continuous Markov process in patch-clamp experiments.

In Eq. 5, a comparison is made between the experimental parameters n_{ij} and the estimated $p_{i,j}(\hat{\phi})$. At a first glance, this seems to be a circulus vitiosus, since also the $p_{i,j}(\hat{\phi})$ have to be calculated from the time series via the measured n_{ij}. However, the assumed model enters the calculation of the $p_{i,j}(\hat{\phi})$ via the relationship between the (macro-channel) probabilities $p_{i,j}$ and the (single-channel) parameters ϕ_l (*see* the examples below). When these relationships are introduced into Eq. 1*b*, and the experimental n_{ij} are used as exponentials, then the determination of the maximum-likelihood estimator $\hat{\phi}$ leads to a relationship between the $p_{i,j}$ and the n_{ij} that is model-dependent. Two concrete examples for the evaluation of $T = T_{ap}$ are given below.

We denote by χ^2_r a χ^2 distribution with r degrees of freedom. The main theorem regarding T_{th} of Eq. 5 (whose proof is sketched in Appendix 1) is

THEOREM 1.

Assume the above model and additionally certain conditions on the hypothesis H as specified in Appendix 1. Further let d (the number of elements of the $m \times m$ transition matrix $P(\phi)$, $\phi \in \Phi$, which are not equal to zero) not depend on ϕ. Then, for every Markov chain with transition matrix $P = P(\phi) \in H$ which generates the data x

$$T^n_{\mathrm{th}}(x) \xrightarrow{d}_n \chi^2_r \tag{6}$$

with $r = d - m - s$.

From Theorem 1 we obtain the following χ^2 test for hypothesis H with fixed error probability α: This test rejects the hypothesis if $T_{th}(x) > q$ with q the α-quantile (i.e., $P(\chi^2_r > q) = \alpha$). Otherwise, i.e., $T_{th}(x) \leq q$, the test accepts the hypothesis. Under every $P \in H$, the probability of rejecting H is asymptotically α.

Substitution of T_{th} by $T = T_{ap}$

Instead of T_{th} we use a test statistic that excludes rare transitions (frequency less than $c \in \mathbb{R}_{>0}$ fixed)

$$T := T_{ap} = T^n_{ap}(x) := \sum_{i,j=1}^{m} \frac{[n_{ij} - n_i p_{i,j}(\hat{\phi})]^2}{n_i p_{i,j}(\hat{\phi})} \times 1_{\max(n_{ij}, n_i p_{i,j}(\hat{\phi}))>c.} \quad (7)$$

The function $1_{\max(n_{ij}, n_i p_{i,j}(\hat{\phi}))>c}$ is 1 if $\max(n_{ij}, n_i p_{i,j}(\hat{\phi})) > c$ and 0 otherwise. Using experience with the χ^2 test for independent, identically distributed random variables it is reasonable to set $c \approx 1$ (Sachs, 1997). We use $c = 1$, throughout the paper.

As reference for our test we do not apply a χ^2 distribution with r degrees of freedom but a χ^2 distribution with

$$r_{ap} = \#\{(i,j) \in \{1,\dots,m\} \times \{1,\dots,m\} : \max(n_{ij}, n_i p_{i,j}(\hat{\phi}))c >\} - m - s \quad (8)$$

degrees of freedom (with the number of elements of a set S denoted by $\#S$). The new test with the new test statistic T and the new reference distribution will be called a χ^2_{ap} test. Notice that r_{ap} is a random variable, i.e., it depends on the measured data sequence. The new test statistic is asymptotically equivalent to the old one of Eq. 5 since $n_i p_{i,j}(\hat{\phi}) \to \infty$ for $n \to \infty$. If Δt depends on n (e.g., $\frac{(k_{ij}\Delta t)^M}{n} \to 0$, for $M \in \mathbb{N}$ fixed, $K = (k_{i,j})_{i,j \in \{1,..,m\}}$ matrix of rate constants) it is possible to get a different asymptotic number of degrees of freedom (but also fixed and not random). (This result is not yet theoretically proved. However, the proof is assumed to be on the lines of the proof of Theorem 1.)

The test statistic T is more adequate for finite observation times than T_{th}: The theoretical number of transitions from i to j under H is approximately (for large n) $n_i p_{i,j}(\hat{\phi})$, close to the observed number n_{ij}. If both values are sufficiently small, i.e., $\max(n_{ij}, n_i p_{i,j}(\hat{\phi})) \le c$, the related pair (i,j) is excluded from the summation. This is sensible since the approximation of the expression $\frac{n_{ij} - n_i p_{i,j}(\hat{\phi})}{\sqrt{n_i p_{i,j}(\hat{\phi})}}$ by a normal distribution (which leads to the χ^2 distribution for T_{th}) is only valid if the values $n_i p_{i,j}(\hat{\phi})$ and n_{ij} are not too small. We make amends for the exclusion of certain pairs (i, j) by reducing the number of degrees of freedom by the same amount.

This substituting procedure is especially important if $p_{i,j}(\hat{\phi})$ is very small such that $n_i p_{i,j}(\hat{\phi}) \approx 0$. Then under H in most cases n_{ij} will be zero which results in

$$\frac{[n_{ij} - n_i p_{i,j}(\hat{\phi})]^2}{n_i p_{i,j}(\hat{\phi})} = n_i p_{i,j}(\hat{\phi}) \approx 0.$$

This term of the sum in Eq. 7 is therefore approximately zero and contributes no degree of freedom for the χ^2 distribution. Therefore the theoretical value for the number of degrees of freedom has to be reduced as done in the χ^2_{ap} test. If under the condition $n_i p_{i,j}(\hat{\phi}) \approx 0$ the unlikely case $n_{ij} > 0$ occurs with $n_{ij} \le c$ then

$$\frac{[n_{ij} - n_i p_{i,j}(\hat{\phi})]^2}{n_i p_{i,j}(\hat{\phi})} \approx \frac{n_{ij}^2}{n_i p_{i,j}(\hat{\phi})}.$$

This very high value results in a very large T_{th}, hence resulting in the wrong decision of the test. It is excluded in the calculation of T.

Remark: The LRT statistic corresponding to the χ^2 statistic T_{th} is

$$T_{LRT} = \sum_{\substack{i,j=1 \\ p_{i,j}(\hat{\phi})>0}}^{m} n_{ij} \log \frac{n_{ij}}{n_i p_{i,j}(\hat{\phi})}.$$

Therefore, for small values of $n_i p_{i,j}(\hat{\phi})$ the same problems arise for T_{LRT} as for T_{th} since the values in the denominator become very small.

In our models for ion channels we use a discrete-time Markov chain. Therefore it is possible that double (triple, ...) jumps occur between time n and $n + 1$. Such simultaneously multiple jumps are impossible in the continuous-time model. The changed test statistic takes care of this problem: If the rate constants in the 2CM are small, multiple jumps are extremely improbable and $n_i p_{i,j}(\hat{\phi}) \approx 0$. This is exactly the case considered above: If the old χ^2 test were used, the number r of degrees of freedom of the reference distribution would not be correct. If the rate constants in the 2CM are large, multiple jumps are possible and we get $T_{th} = \mathrm{T}_{ap} = \mathrm{T}$ and $r = r_{ap}$. It is possible to treat the cases of multiple jumps in a different kind of way by letting the sampling interval Δt depend on n as indicated above (below Eq. 8). Then an approximation of P by the first M terms of the Taylor expansion in Eq. 2 is possible.

For the models discussed below, the above theorem leads to the following strategy explained for a patch-clamp record with two conductivity levels. It has to be tested whether this record is caused by two channels or by one channel with a substate. The $p_{i,j}$ are the transition probabilities of the macro-channel, that is a putative channel comprising all states of the assumed scenarios. On the basis of the hypothesis of two independent channels, the $p_{i,j}$ can be calculated from the ϕ_l, the transition probabilities of the individual channels (*see* below, or Blunck et al., 1998). The n_{ij} and the n_i of Eqs. 5 and 7 are obtained from the measured time series by a jump detector, and the $p_{i,j}(\hat{\phi})$ are determined as described above by means of Eqs. 1*b* and 3. After computing T, a χ^2_{ap} test can be performed.

Theorem 1 states the behaviour of the test statistic T_{th} in the case that the underlying but unknown transition matrix P is in H. It does not provide any statement if P is in the alternative H^C. Especially, no probability is given for the truth of H (or H^C) if $T_{th}(x) \leq q$. The behaviour of T under the alternative is described in the next theorem (the proof is straightforward, *see* Caliebe, 1996):

THEOREM 2.

We assume the conditions of Theorem 1. Let $P \in H^C$. Let κ be a constant vector of $\mathbb{R}^s$ such that

$$\hat{\phi} \xrightarrow{p}_n \kappa. \tag{9}$$

Then there exists a constant $\gamma \in \mathbb{R}_{>0}$ such that

$$\frac{T}{n} \xrightarrow{p}_n \gamma. \tag{10}$$

This means that if the alternative ($P \in H^C$) is true, and if Eq. 9 is satisfied, the test statistic T shows an asymptotic linear increase with the number of data points. T exceeds every given boundary for sufficiently large n. Our proposed χ^2_{ap} test will then reject the hypothesis.

Basic Models for Simulations

Simple Two-Channel Model

$$\begin{aligned} &\mathrm{C} \rightleftharpoons \mathrm{O} \\ &\mathrm{C}' \rightleftharpoons \mathrm{S} \end{aligned} \tag{11}$$

In the simple two-channel model (2CM) of Eq. 11, independence of the channels is always assumed (Yeo et al., 1989; Dabrowski, McDonald & Rösler, 1990; Dabrowski & McDonald, 1992). It is assumed that the current of state O is different from the current of state S. The resulting macro-model can be viewed as a Markov chain with four states, which will be numbered from one to four as shown in Table 1. The free parameters ϕ_l are the transition probabilities of the two single channels:

$$\phi_1 = p_{C,O} \quad \phi_2 = p_{O,C} \quad \phi_3 = p_{C',S} \quad \phi_4 = p_{S,C'} \tag{12}$$

with $0 < \phi_l < 1, l = 1, 2, 3, 4$.
Recall that $p_{C,C} = 1 - p_{C,O}$, $p_{O,O} = 1 - p_{O,C}$, etc.. Therefore, the transition probabilities $p_{C,C}$, $p_{O,O}$, $p_{C',C'}$, and $p_{S,S}$ are not free parameters.

For the assumed model the theoretical transition matrix of the macro-channel $P(\phi)$ can then be calculated in terms of the vector $\phi = (\phi_1, \phi_2, \phi_3, \phi_4)$ of single-channel parameters (by virtue of the independence of the channels):

$$\begin{aligned} p_{1,1}(\phi) &= p_{C,C}p_{C',C'} = (1-\phi_1)(1-\phi_3) \\ p_{1,2}(\phi) &= p_{C,C}p_{C',S} = (1-\phi_1)\phi_3, \text{etc.} \end{aligned} \tag{13}$$

Table 1. Assignment of the biological states of two superimposed single channels of Eq. 11 to the macrochannel states of the resulting Markov chain

Biological State	Mathematical state
(C,C′)	1
(C,S)	2
(O,C′)	3
(O,S)	4

Here letters as indices denote the single-channel states and numbers as indices the states of the macro-channel. To calculate the test statistic T of Eq. 7 one has to proceed as follows: The maximum-likelihood estimators $\hat{\phi}_l$ are obtained from Eq. 4 with L from Eq. 1*b* and the $p_{i,j}$ taken from Eq. 13. Thus

$$\pi(x_0)\frac{\partial}{\partial \phi_l}\prod_{i,j=1}^{4} p_{i,j}(\phi)^{n_{i,j}} = 0 \tag{14}$$

with $l = 1, 2, 3, 4$ results in

$$\begin{aligned} \hat{p}_{C,O} &= \hat{\phi}_1 = \frac{n_{12}+n_{14}+n_{32}+n_{34}}{n_1+n_3}, \\ \hat{p}_{O,C} &= \hat{\phi}_2 = \frac{n_{21}+n_{23}+n_{41}+n_{43}}{n_2+n_4}, \\ \hat{p}_{C',S} &= \hat{\phi}_3 = \frac{n_{13}+n_{14}+n_{23}+n_{24}}{n_1+n_2}, \\ \hat{p}_{S,C'} &= \hat{\phi}_4 = \frac{n_{31}+n_{32}+n_{41}+n_{42}}{n_3+n_4}, \end{aligned} \tag{15}$$

Introducing the n_{ij} from the data and the $\hat{\phi}_l$ of Eq. 15 into Eq. 13 and Eq. 7 yields the numerical value of T, and a χ^2_{ap} test can be performed.

Two-Channel Model with Double Number of Channels

The following model

$$\begin{aligned} &2 \times \mathrm{C} \rightleftharpoons \mathrm{O} \\ &2 \times \mathrm{C}' \rightleftharpoons \mathrm{S} \end{aligned} \tag{16}$$

results in a Markov chain (macro-channel) of nine states as shown in Table 2. The parameter vector ϕ is the same as in the simple 2CM above (Eq. 12). Because of the double number of channels, each element of the transition matrix of the macro-channel is now a product of four factors:

$$\begin{aligned} p_{1,1}(\phi) &= p_{C,C}p_{C,C}p_{C',C'}p_{C',C'} = (1-\phi_1)^2(1-\phi_3)^2 \\ p_{1,2}(\phi) &= 2p_{C,C}p_{C,O}p_{C',C'}p_{C',C'} \\ &= 2(1-\phi_1)\phi_1(1-\phi_3)^2, \text{etc.} \end{aligned} \tag{17}$$

(As before in Eq. 13, indices with letters denote the single-channel states and indices with numbers the states of the macro-channel.)

Substituting these expressions in Eq. 1*b* and in the maximum-likelihood equations, Eq. 4 results in a

Table 2. Assignment of the biological states of four superimposed single channels of Eq. 16 to the macrochannel states of the resulting Markov chain

Biological State	Mathematical state
(C,C,C′, C′)	1
(C,O,C′,C′)/(O,C,C′,C′)	2
(C,C,S,C′)/(C,C,C′,S)	3
(C,O,C′,S)/(O,C,C′,S)/(C,O,S,C′)/(O,C,S, C′)	4
(O,O,C′,C′)	5
(C,C,S,S)	6
(O,O,C′,S)/(O,O,S,C′)	7
(C,O,S,S)/(O,C,S,S)	8
(O,O,S,S)	9

polynomial in $\hat{\phi}_1$ (resp. $\hat{\phi}_2$, $\hat{\phi}_3$ and $\hat{\phi}_4$) of order five with the coefficients being determined by the measured n_{ij} . These polynomials have to be solved numerically in order to get $\hat{\phi}$ for Eq. 7. In most cases, however, when the rate constants (and therefore the transition probabilities) are small with respect to the sampling frequency of the data, an approximation is possible: Quadratic expressions in ϕ_l can be ignored in comparison with linear terms in ϕ_l provided that the respective coefficients are of the same magnitude. This yields much simpler expressions for the $\hat{\phi}_l$, which are now the solutions of a quadratic equation (*see* Appendix 2). By means of Eq. 7 the assumed model can be tested.

Simulations

In all simulations (based on a program of Riessner, 1998; Blunck et al., 1998), a sampling rate of 200 msec^{-1} was used.

Validity of the Asymptotic Statements

We considered the simple 2CM of Eq. 11 as our hypothesis. The appropriate macro-channel model is shown above (Table 1).

Behavior of the Test under the Hypothesis

Under the hypothesis (i.e., the assumed model is correct) the test value T is asymptotically χ^2 distributed according to Theorem 1. However, this distribution is only guaranteed for a large number n of observations, i.e., for a sufficiently high number of transitions in a measured time series. In a typical experiment, the number of data points is about 10^6. In the following simulations it was tested whether the asymptotic χ^2 distribution is already obtained by this number. This would ensure that the test can be applied to such records. Note that because of only finitely many observations, the number of degrees of freedom of the reference χ^2 distribution of the χ^2_{ap}

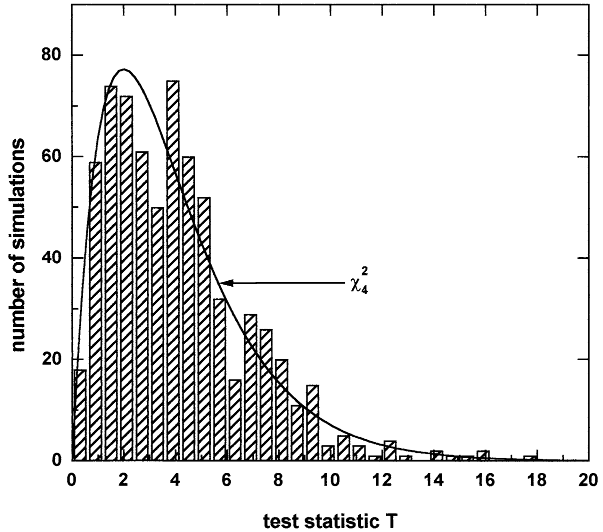

Fig. 1. Distribution of simulated values of the test statistic of the model of Eq. 18 obtained from 700 simulations with 10^6 data points each tested with 2CM hypothesis. The smooth line shows a χ^2_4 distribution.

test, r_{ap}, may differ from r, the number of degrees of freedom of the reference χ^2 distribution of the standard χ^2 test.

For our simulations we used a 2CM with rate constants as follows:

$$C \underset{5sec^{-1}}{\overset{10sec^{-1}}{\rightleftharpoons}} O$$

$$C' \underset{60sec^{-1}}{\overset{40sec^{-1}}{\rightleftharpoons}} S \quad (18)$$

The appropriate macro-channel model is shown above (Table 1). In all our simulations we had $\max(n_{ij}, n_i p_{i,j}(\hat{\phi})) \ll c = 1$ for all pairs (i,j) that correspond to double jumps (the pairs (1,4), (2,3), (3,2) and (4,1)). This is due to the comparatively small rate constants of the model. Double jumps are highly improbable in this setting. Therefore

$$\begin{aligned} r_{ap} = \#\{(i,j) \in \{1,\ldots,m\} \\ \times \{1,\ldots,m\} : \max(n_{ij}, n_i p_{i,j}(\hat{\phi})) > c\} \\ - m - s = 12 - 4 - 4 = 4 \end{aligned} \quad (19)$$

for all measured observation sequences.

Fig. 1 shows the distribution of the test statistic T obtained from 700 simulations. The simulated T values for 10^6 data points (individual bars in Fig. 1) approximate the theoretical χ^2 distribution (smooth line in Fig. 1 — recall that the maximum of a χ^2 distribution of l degrees of freedom is at the abscissa value $l - 2$).

The difference between r and r_{ap} resulted from the very small probability of double jumps. If the corresponding values $\max(n_{ij}, n_i p_{i,j}(\hat{\phi}))$ exceed the limit c, then r_{ap} will take the full value $m^2 - m - s$. The influence of double jumps on the number of degrees of freedom of the χ^2 distribution is illustrated by simulations with rate constants that are a thousand times faster than those used in Eq. 18. Now, double jumps are possible and $\max(n_{ij}, n_i p_{ij}(\hat{\phi})) > c$ for the corresponding pairs. We obtain

$$r_{ap} = m^2 - m - s = 16 - 4 - 4 = 8. \tag{20}$$

Fig. 2 shows that the experimental distribution can be fitted by the theoretical χ^2 distribution. Only 100 simulations were necessary for approximating the theoretical curve because the higher rate constants resulted in more jumps and thus in a lower scatter of the value of the test statistic of the individual records.

Remark: The 2CM of Eq. 18 should result in the same χ^2 distribution with 8 degrees of freedom if the number of sampling points is multiplied by a factor 10^6 (the factor $(10^3)^2$ results from the fact that the $p_{i,j}$ for double jumps are the product of two single-channel transition probabilities). Then, the low probability for double jumps would be compensated by a very long observation time.

These results show that for a realistic number of data points (10^6) the test can be applied. This is even true for rate constants as small as those in Eq. 18, resulting in only a few transitions between different states.

For the demonstration of the behavior of the test statistic for fewer data points one can inspect the development of the test statistic with the number of data points in Fig. 3 (data generated by the 2CM). The test statistic remains always below the threshold. Therefore, even for a small number of observations (about 10 jumps at 50,000 samples), the test gives the correct decision: If T is below the threshold of the χ^2_{ap} test, the 2CM is chosen. If the number of observations was further reduced such that only about 4 jumps were recorded, the test statistic nearly always was zero. In such a case of too few observable events, it makes no sense to perform a χ^2_{ap} test because obviously the approximating χ^2 distribution does not apply.

Behavior of the Test under the Alternative

In the case of the alternative (the 2CM is false, i.e., the data is generated by a SLM) there exists no statement concerning the distribution of the test statistic. Theorem 2, however, yields an alternative approach, based on an asymptotic linear increase of T with the number of data points. The following SLM was used to investigate whether this linear increase was found at a realistic number of data points:

$$C \underset{400\ \mathrm{sec}^{-1}}{\overset{20\ \mathrm{sec}^{-1}}{\rightleftharpoons}} O \underset{1000\ \mathrm{sec}^{-1}}{\overset{1000\ \mathrm{sec}^{-1}}{\rightleftharpoons}} S \tag{21}$$

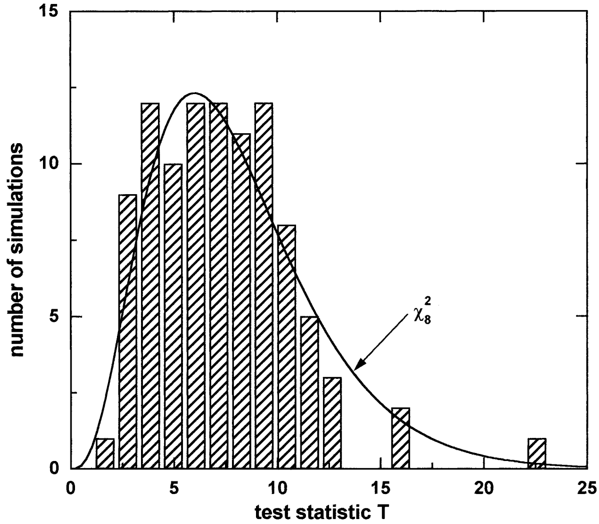

Fig. 2. Distribution of simulated values of the test statistic of the model of Eq. 18 with rate constants multiplied by 1000 obtained from 100 simulations with 10^6 data points, each tested with the 2CM hypothesis. The smooth line shows a χ^2_8 distribution.

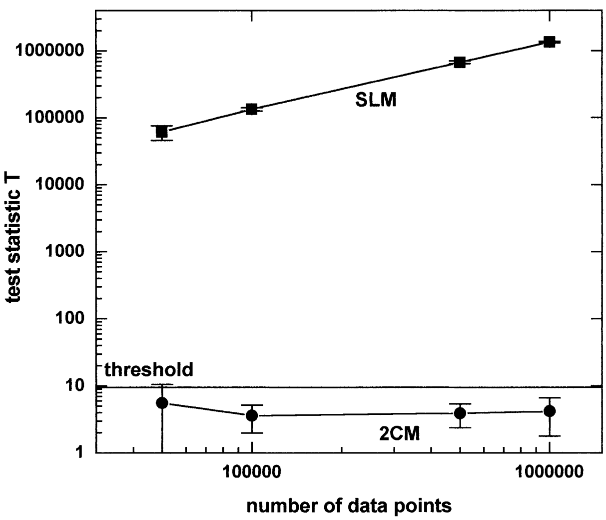

Fig. 3. Development of the test statistic with the number of data points for data generated by the 2CM of Eq. 18 (circles) and by the SLM of Eq. 21 (squares) tested with 2CM hypothesis. For each number of data points 5 simulations were made for the 2CM and for the SLM. Error bars show the standard deviation. The horizontal line gives the threshold for a χ^2_{ap} test of a reliability of 95 % if the 2CM is rejected.

In Fig. 3 (data generated by the SLM) the postulated linear increase can be clearly seen even for a low number of data points. Furthermore, the values of T are very high and lie several magnitudes above the threshold: The test gives the correct decision. The 2CM is rejected with a probability of 95 % (even with higher probability if a different error value had been chosen resulting in a higher threshold).

Distinction of Similar Models

Non-equidistant Current Levels

$$\begin{matrix} C \rightleftharpoons O \\ C' \rightleftharpoons S \end{matrix} \iff S \rightleftharpoons C \rightleftharpoons O \qquad (22)$$

for $k_{C,O}$, $k_{C,S}$, $k_{C',S}$ small.

In the model of Eq. 22, it is assumed that the current of the open state O is clearly different from the current of the open state S.

The special problems of the models of Eq. 22 as compared to those of Eq. 21 and Eq. 18 are as follows:

- In the model of Eq. 21, transitions between S and O (state 2 and state 3 of the Markov chain/macrochannel in Table 1) can be used to distinguish between the models: They are frequent in the SLM due to relatively high rate constants and rare in the 2CM because they have to be created by highly improbable double jumps. These transitions are forbidden in the SLM of Eq. 22, and thus their non-occurrence is a common feature of both models of Eq. 22, and cannot serve as a means of distinction between the models.
- In the SLM of Eq. 21 the exclusion of transitions between C and S as compared to the 2CM is a tool for distinguishing between the models: However, in the SLM of Eq. 22 they are allowed as well.
- The state O + S (state 4 of the Markov chain, Table 1) must not occur in the SLM of Eq. 21, and thus its occurrence in a time series is a strong indication of the 2CM. This also applies to the models in Eq. 22. However, also this criterion can loose its power if an unfavorable set of rate constants is given (rate constants of opening being much smaller than those of closing), which keep the occurrence of this state low also in the 2CM.

Thus, a simple criterion for excluding models is not available in the case of Eq. 22. Therefore, it is investigated whether the calculation of the test statistic may be helpful. In this case our hypothesis is the 2CM of Eq. 22. The considerations regarding the occupation of state O + S draw the attention to the ratio between opening and closing rate constants. The influence of this parameter is displayed in Fig. 4. If the probability for closing is 1000 times higher than that for opening, the models cannot be distinguished by means of the test statistic (10^6 data points): With respect to the test statistic of the data set generated by the SLM, a 2CM is also possible. This seems to originate from the situation discussed above: If data were generated by a 2CM that has the same rate constants as the SLM, then the occupation of state O + S would be so rare that it would not provide a means of distinguishing the models of Eq. 22. If the values of the rate constants become more similar, the test of data generated by the SLM of Eq. 22 rejects the 2CM. This shows that the test is relatively sensitive, because the rate constants for closing and opening may differ by a factor of 100 and the test is still able to reject the 2CM if the SLM holds.

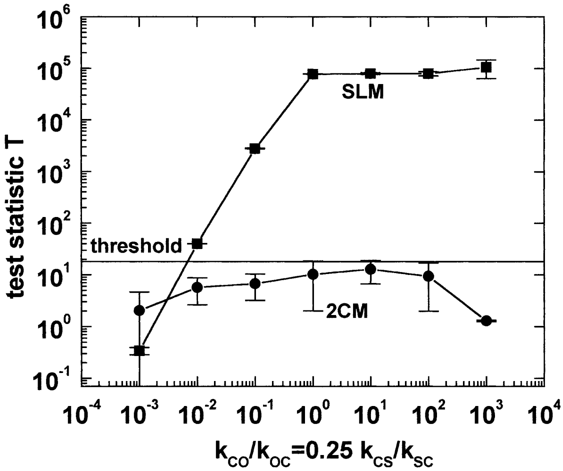

Fig. 4. Development of the test statistic with increasing ratio between opening and closing rate constants for data generated by the 2CM (circles) and by the SLM (squares) of Eq. 22 tested with the 2CM hypothesis. For each ratio 3 simulations of 10^6 data points were made for the 2CM and for the SLM. Error bars show the standard deviation. The horizontal line gives the threshold for a χ^2_{ap} test of a reliability of 95% if the 2CM is rejected.

In the case of a patch-clamp record without the occurrence of the state O + S, the observer is apt to assume an underlying SLM. It is not clear, however, whether a 2CM with small open probabilities might be possible as well. The χ^2_{ap} test is able to reject the 2CM with a preset probability.

There is another means of distinguishing models even when the test value T itself fails: the linear increase of T with the number of data points. For the demonstration of this approach, the model of Eq. 22 is used with the following rate-constants

$$\begin{matrix} C \underset{10000\ \text{sec}^{-1}}{\overset{100\ \text{sec}^{-1}}{\rightleftharpoons}} O \\ C' \underset{10000\ \text{sec}^{-1}}{\overset{400\ \text{sec}^{-1}}{\rightleftharpoons}} S \end{matrix} \iff S \underset{400\ \text{sec}^{-1}}{\overset{10000\ \text{sec}^{-1}}{\rightleftharpoons}} C \underset{100\ \text{sec}^{-1}}{\overset{10000\ \text{sec}^{-1}}{\rightleftharpoons}} O \qquad (23)$$

Fig. 5 shows the result: If the models were simulated with a small number of data points (less than 500,000), they cannot be distinguished by means of the test statistic T. Only after sufficiently long observation time, enough information is collected to reject the 2CM for data generated by the SLM (which can mainly be viewed as the non-occurrence of state O + S). However, the linear increase of the test statistic for data generated by the SLM is striking. It occurs even in the region where the test value alone cannot decide which model is correct. This linear increase can be used as a strong indication that the hypothesis is false.

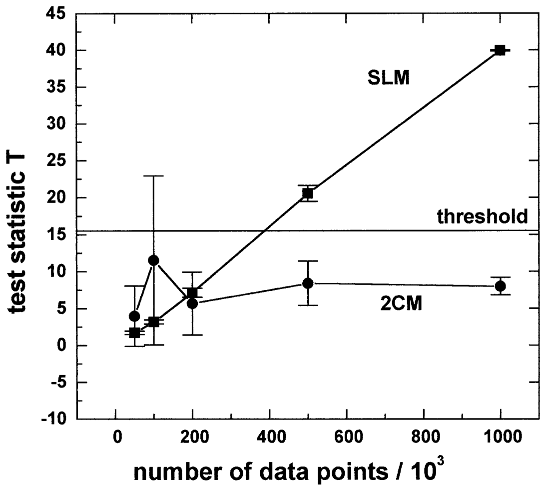

Fig. 5. Development of the test statistic with the number of data points for data generated by the 2CM (circles) and by the SLM (squares) of Eq. 23 tested with 2CM hypothesis. The average observed number of transitions from O to S is near the values of the test statistic for the SLM. For each number of data points 3 simulations were made for the 2CM and for the SLM. Error bars show the standard deviation. The horizontal line gives the threshold for a χ^2_{ap} test of a reliability of 95 % if the 2CM is rejected.

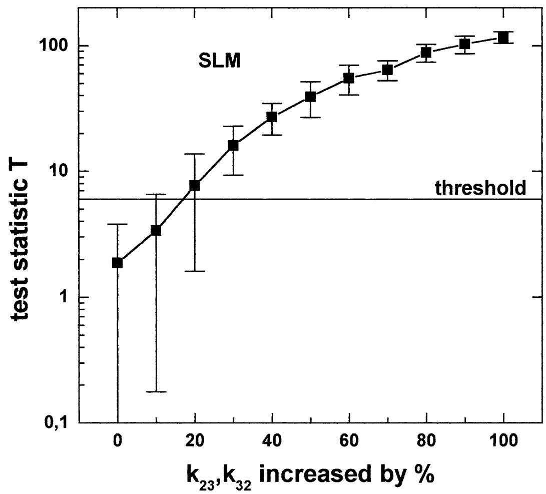

Fig. 6. Dependence of the test statistic on the deviation of the rate constants $k_{O,2O}$ and $k_{2O,O}$ from those of a SLM, which is equivalent to the 2CM of Eq. 26 (for the equivalence, *see* Eq. 25), tested with 2CM hypothesis. For each value of the rate constants 5 simulations of the SLM were made. Error bars show the standard deviation. The horizontal line gives the threshold for a χ^2_{ap} test of a reliability of 95% if the 2CM is rejected.

Equidistant Current Levels

$$2 \times \mathrm{C} \rightleftharpoons \mathrm{O} \iff \mathrm{C} \rightleftharpoons \mathrm{O} \rightleftharpoons 2\mathrm{O} \tag{24}$$

In the model of Eq. 24, it is assumed that in the SLM the current of the open state O is half the current of the open state 2O. For sake of clarity, double jumps are ignored so that transitions from C to 2O are impossible. It is no problem, however, to incorporate double jumps into the models.

The model of Eq. 24 is of special interest: In the model of the previous section it was possible to decide in favour of the 2CM if the state O + S occurs. This is not a means of distinguishing between the models here, because the states C, O and 2O may occur in both SLM and 2CM.

Note that the following two models are kinetically equivalent because of the special choice of the transition probabilities:

$$2 \times \mathrm{C} \underset{p_{OC}}{\overset{p_{CO}}{\rightleftharpoons}} \mathrm{O} \iff \mathrm{C} \underset{p_{CC}p_{OC}}{\overset{2p_{CC}p_{CO}}{\rightleftharpoons}} \mathrm{O} \underset{2p_{OC}p_{OO}}{\overset{p_{CO}p_{OO}}{\rightleftharpoons}} 2\mathrm{O} \tag{25}$$

It is therefore impossible to distinguish between the two models of Eq. 25 by any test. It is interesting, however, to test the 2CM against a SLM with rate constants between O and 2O slightly different from those of the SLM of Eq. 25. The following 2CM was investigated:

$$2 \times \mathrm{C} \underset{70\ \mathrm{sec}^{-1}}{\overset{100\ \mathrm{sec}^{-1}}{\rightleftharpoons}} \mathrm{O} \tag{26}$$

In comparison with the 2CM of Eq. 24 we tested a SLM with rate constants $k_{O,2O}$ and $k_{2O,O}$ increased by 10 to 100% as compared to the SLM of Eq. 25. The 2CM of Eq. 24 was used as hypothesis. The result is shown in Fig. 6: If the rate constants are 20% or more higher than those of the equivalent SLM of Eq. 25, the test is able to distinguish between the models by rejecting the 2CM. This shows high sensitivity of the test to compare the models of Eq. 24.

As in the previous section, in the region where the test statistic T itself cannot be used to reject the SLM, the linear increase of T with the sampling time in the region of more than 10^5 observations gives a strong indication for the SLM. This is displayed in Fig. 7 for $k_{O,2O}$ and $k_{2O,O}$ increased by 40%.

Application to Noisy Data

In patch-clamp measurements, the observed data are usually disturbed by noise, and the original time series has to be reconstructed by means of a jump detector. Noise and limited temporal resolution can lead to three kinds of errors introduced by the jump detector: false alarms, when noise initiates a transition that does not occur in the original Markov process, missed events, if the events are too short for the detector or noise compensates a real transition of the Markov process, and false jumps, when a false target is assigned to an actual jump.

Behavior of the Test with Increasing Noise

Time series were generated by the 2CM of Eq. 18 and superimposed by white noise, whose magnitude, is described by the signal-to-noise ratio

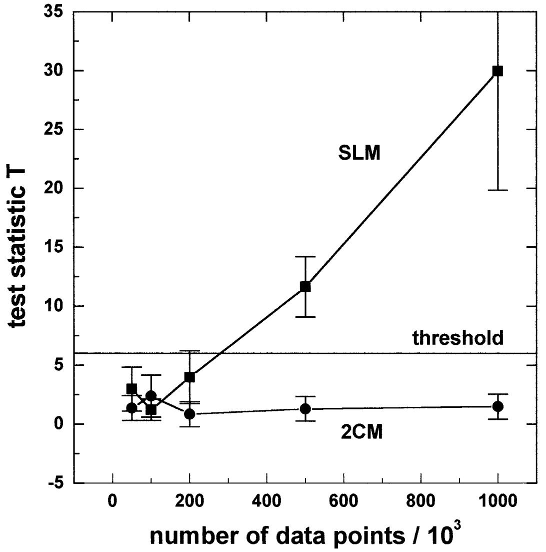

Fig. 7. Development of the values of the test statistic with the number of data points for data generated by the 2CM (circles) and by the SLM (squares) of Eq. 24 tested with 2CM hypothesis. The rate constants $k_{O,2O}$ and $k_{2O,O}$ of the SLM are increased by 40 % as compared to the SLM of Eq. 25. For each number of data points 5 simulations were made for the 2CM of Eq. 26 and for the SLM. Error bars show the standard deviation. The horizontal line gives the threshold for a χ^2_{ap} test of a reliability of 95 % if the 2CM is rejected.

$$\mathrm{SNR} = \frac{\Delta I}{\sigma} \tag{27}$$

with σ being the standard deviation of the noise and ΔI being the smallest current difference of the investigated levels. The test was performed with the hypothesis of a 2CM. Figure 8 shows that up to an SNR of 2 the test statistic stays below the threshold such that the test still gives the correct result. With an SNR of 1.7 or less, the data are so much disturbed that the test rejects the assumed model. The test can therefore be applied up to an SNR of two. This is a reasonable result because most analyzing techniques cannot be used for an SNR lower than 2.

Noise Correction for a Low SNR

For the understanding of why the test does not give the correct result when the SNR is too low and for the construction of a correction algorithm, the two transition matrices of Table 3 are inspected: The occurrence of $n_{23} = 2$ and $n_{32} = 2$ in matrix B (which gives the wrong test statistic, high noise) attracts attention. These transitions are zero in matrix A (low noise). The simulation program offers the option to print out the original transition matrix of the simulated time series of matrix B, which is obtained from the Markov process before noise is added. In this matrix, there is $n_{23} = 0$ and $n_{32} = 0$ as in matrix A. Obviously, the 2s are false alarms or false jumps

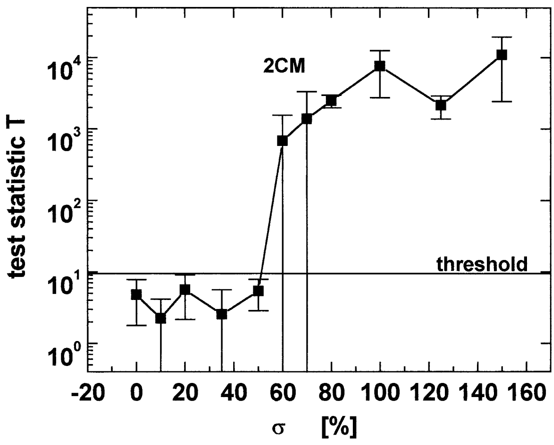

Fig. 8. Dependence of the test statistic on noise for data generated by the 2CM of Eq. 18 tested with 2CM hypothesis. σ is the standard deviation of the superimposed white noise given in percent of the smallest current difference ΔI of the investigated levels. For each σ value 3 simulations of the 2CM were made. Error bars show the standard deviation. The horizontal line gives the threshold for a χ^2_{ap} test of a reliability of 95 % if the 2CM is rejected.

Table 3. Typical transition matrices for simulations with low and high noise. Matrix A was obtained from a simulation with A σ OF 10 % and matrix B with A σ of 70 %. The standard deviation σ of the white noise is given in percent of the smallest current difference ΔI of the investigated levels. The transitions $N_{23} = 2$ and $N_{32} = 2$ In matrix B were caused by an error of the jump detector due to noise

	A	Sink State			
		1	2	3	4
Source State	1	217591	10	38	0
	2	6	376995	0	74
	3	41	0	142633	6
	4	0	71	9	262525
	B	Sink state			
		1	2	3	4
Source State	1	243414	12	38	0
	2	10	397591	2	80
	3	39	2	114070	6
	4	0	78	8	244654

generated in the detector by noise. Further simulations gave the same result: a failure of the test statistic occurred when n_{23} and/or n_{32} were not equal to 0. If these wrong values were removed artificially, the test resulted in the correct decision.

The crucial role of n_{23} and n_{32} is not unexpected because such transitions are highly improbable in the 2CM of Eq. 18 since low rate constants practically exclude double jumps.

If an algorithm were available that yields a prediction of how many of the detected jumps result from noise then a correction could be applied to get the transition matrix of the noise-free records. Such

Table 4. Expected values of false jumps between level 2 and 3 of the model of Eq. 18 as caused by noise

σ [%]	SNR	n_{23}^{sim}	$E(n_{23}^{th})$	$\sigma(n_{23}^{th})$
10	10	0	0	0
20	5	0	10^{-19}	10^{-10}
35	2.86	0	0.417	0.638
50	2	0	0.605	0.763
60	1.67	$\frac{1}{3}$	0.548	0.730
70	1.43	$\frac{2}{3}$	0.590	0.758
80	1.25	$1\frac{2}{3}$	0.649	0.796
100	1	$\frac{2}{3}$	0.598	0.765
125	0.8	$1\frac{1}{3}$	0.569	0.748
150	0.67	$2\frac{1}{3}$	0.704	0.832

The standard deviation σ of the white noise is given in percent of the smallest current difference ΔI of the investigated levels. The SNR is computed according to Eq. 27. n_{23}^{sim} is obtained from the average of 3 simulations with superimposed white noise. $E(n_{23}^{th})$ and $\sigma(n_{23}^{th})$ are the expected value and standard deviation of false jumps N23 calculated theoretically as described in Appendix 3 (Eqs. A3.8–A3.10).

an algorithm can be created using the special structure of the Hinkley detector (Schultze & Draber, 1993, employed here for the evaluation of the time series). The calculations for the model in Eq. 18 by means of Eqs. A3.8–A3.10 in Appendix 3 lead to the theoretically expected values of false jumps n_{23} from state 2 to 3 ($E(n_{23}^{th})$) and their scatter ($\sigma(n_{23}^{th})$) as shown in Table 4 for different SNRs (calculation relating to n_{32} can be done using the same method). The comparison with the column n_{23}^{sim} obtained from the average of three simulations shows that the prediction of false jumps is correct for an SNR down to 0.8, i.e., $n_{23}^{sim} \in [E(n_{23}^{th}) - \sigma(n_{23}^{th}), E(n_{23}^{th}) + \sigma(n_{23}^{th})]$. This means that measured values of n_{23}, which are in the above range given by $E(n_{23}^{th})$ and $\sigma(n_{23}^{th})$, can be set to zero.

Simulations for Multiple Identical Channels

The following model was tested to check the applicability of the test for models with multiple identical channels:

$$\begin{array}{ll} 2\times & C \underset{5\ \mathrm{sec}^{-1}}{\overset{10\ \mathrm{sec}^{-1}}{\rightleftharpoons}} O \\ 2\times & C' \underset{60\ \mathrm{sec}^{-1}}{\overset{40\ \mathrm{sec}^{-1}}{\rightleftharpoons}} S \end{array} \tag{28}$$

The appropriate macro-channel was calculated above (Table 2). The number of states m of the Markov chain is 9 and the number of free parameter s is 4. For our simulations we had

$$\#\{(i,j) \in \{1,\ldots,m\} \times \{1,\ldots,m\} \\ : \max(n_{ij}, n_i p_{i,j}(\hat{\phi})) > c\} = 33$$

(as in the case of the simple 2CM, transition probabilities that require double jumps are highly im-

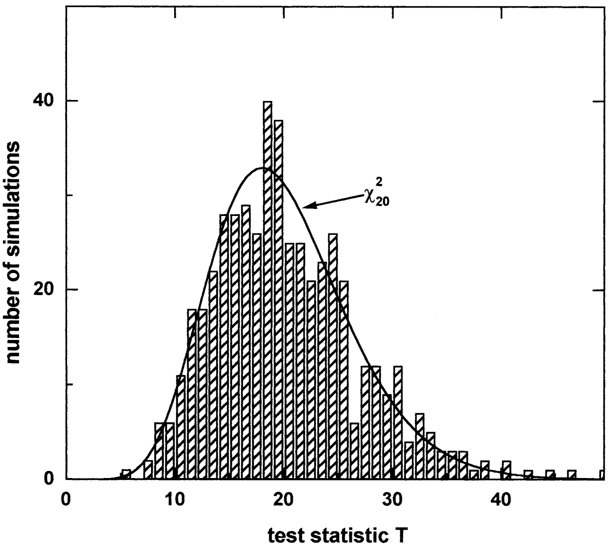

Fig. 9. Distribution of simulated values of the test statistic of the model of Eq. 28 obtained from 500 simulations with 10^6 data points each tested with 2CM hypothesis. The smooth line shows a χ^2_{20} distribution.

probable). We expect therefore a convergence to a χ^2 distribution with

$$r_{ap} = 33 - 9 - 4 = 20 \tag{29}$$

degrees of freedom (Eq. 8) taking the 2CM of Eq. 16 as hypothesis. Fig. 9 shows that this distribution is correctly approximated by the empirical simulated distribution of the test statistic. Thus, the test can also be used for testing complex models such as models with multiple identical channels.

Simulations for Aggregated Markov Chains

Models of ion channels often comprise multiple open and closed states. They are not strict Markov models, but so-called aggregated Markov models because each observed current level cannot be assigned to a single state of the model, as is required for Markov chains. We consider the following example:

$$\begin{array}{l} C_2 \underset{20\ \mathrm{sec}^{-1}}{\overset{100\ \mathrm{sec}^{-1}}{\rightleftharpoons}} C_1 \underset{500\ \mathrm{sec}^{-1}}{\overset{1000\ \mathrm{sec}^{-1}}{\rightleftharpoons}} O \\ \qquad C_3 \underset{60\ \mathrm{sec}^{-1}}{\overset{40\ \mathrm{sec}^{-1}}{\rightleftharpoons}} S \end{array} \tag{30}$$

If only the number of transitions in long time series is observed, and if ergodicity is assumed then the number of transitions of channels from state O to C_1 and vice versa can be considered under steady-state conditions. This implies that in the rate equation of Eq. 30

$$\frac{dO}{dt} = k_{C_1,O}C_1 - k_{O,C_1}O \tag{31}$$

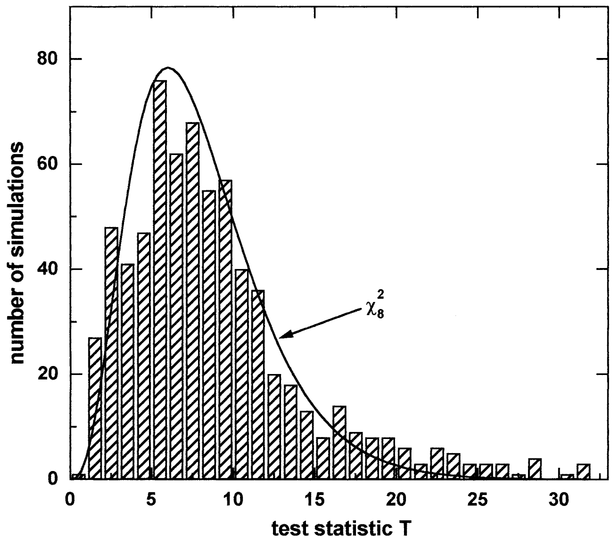

Fig. 10. Distribution of simulated values of the test statistic of the model of Eq. 30 obtained from 700 simulations with 10^6 data points each tested with 2CM hypothesis. The smooth line shows a χ^2_8 distribution.

(with O and C_1 the concentrations of state O and C_1, respectively) C_1 can be replaced by its steady-state concentration C:

$$C_1 = \frac{k_{C_2,C_1}}{k_{C_1,C_2} + k_{C_2,C_1}} C =: r_1 C \tag{32}$$

leading to

$$\frac{dO}{dt} = \frac{k_{C_1,O}}{r_1} r_1 C_1 - k_{O,C_1} O = \kappa_{C,O} C - k_{O,C_1} O \tag{33}$$

with r_1 being the reserve factor (Hansen, Tittor & Gradmann, 1983), which merges all "indistinguishable" states (C_1 and C_2) into a representative state (C). It should be mentioned that C_1 and C_2 become distinguishable states when dwell-time histograms are considered. However, when the transitions n_{ij} in a long time series are counted, as required by the test, the temporal information is lost (Eq. 1*b*), C_1 and C_2 become indistinguishable and the premises for using reserve factors are fulfilled. As a consequence, the transition rates can be treated on the basis of a Markov model.

These considerations are illustrated by means of simulations on the basis of the model of Eq. 30. Fig. 10 shows that the conversion of the aggregated Markov model to a Markov model by reserve factors is legitimate as the simulated distribution of 700 values of the test statistic T approximates a χ^2 distribution of 8 degrees of freedom (2CM hypothesis). The number of 8 results from the fact that the magnitude of the rate constants leads to double jumps.

Discussion

The simulations above show that the length of the time series of usual experiments is sufficient to fulfill the premise of the test, namely, that the test statistic of Eq. 7 is χ^2-distributed. It is obvious that in most cases the test yields a reliable distinction between models. In the case where the situation is unfavourable (not enough data points), the linear increase of the test statistic (Theorem 2) can be used as an indication that the data originate from the alternative model. In most examples, the discrimination of the models depends on the existence of the O + S state, which is forbidden in the SLM. In those cases, the test may be regarded as a tool that provides more comfort, but no new insights, as the occurrence of this state could also be checked by a visual inspection of a sufficiently long time series. However, the C—O—2O model is an example for a scenario where the visual inspection would not help. Both models, 2CM and SLM, would generate 3 levels. The χ^2_{ap} test is capable of revealing the difference in predicted rate constants for the O—2O transition.

Noise is always a serious problem in the analysis of patch-clamp data. The correction algorithm derived on the basis of a Hinkley detector (Eqs. A3.8–A3.10), however, is a means to improve the reliability of the test also for data heavily corrupted by noise.

Most simulations were done on the basis of the simple 2CM or SLM of Eqs. 18 and 21, respectively, as the low number of states reduced computer time for thousands of simulations to a tolerable extent. Another reason was that Theorems 1 and 2 are derived for Markov models and not for aggregated Markov models. However, this is not a serious restriction. The test statistic is not influenced by the temporal sequence of the transitions, and the information obtained in multi-exponential dwell-time distribution is lost (Eq. 1*b*). The theory of reserve factors (Hansen et al., 1983) implies that states in reaction kinetic schemes that cannot be revealed in the actual experiment can be comprised in apparent states. This theoretical expectation is verified by the simulations of the model of Eq. 30: The distribution of the test statistic calculated from Eq. 30 is a χ^2 distribution as in the case of pure Markov models. Further, the test is also applicable to multi-channel records that are presented by macro-models (which are also aggregated Markov models, as different states have equal conductances).

The inclusion of the temporal behavior would utilize more of the information contained in the measured time series. This could be done if another approach for a test is applied (likelihood ratio test). This approach would be based on the calculation of the likelihood of the time series (Fredkin & Rice, 1992*a*; Albertsen & Hansen, 1994; Klein, Timmer &

Honerkamp, 1997), i.e., the likelihood that the observed sequence of dwell-times in the levels of the records has occurred, calculated on the basis of an assumed hidden Markov model. Using this likelihood as a test statistic also leads to a χ^2 distribution (Bickel, Ritov & Rydén, 1998). However, when we tested this procedure by simulations, there was a practical problem. The convergence of the fit routines (in our case a simplex algorithm, Albertsen & Hansen, 1994) is not reliable enough. Every time series requires several fits with different starting values and a test based on the analysis of a time series reconstructed from the evaluated rate constants. For a complicated model in the best case, it may take about one day for a single time series (Farokhi, Keunecke & Hansen, 2000), in the worst case, the routine does not converge at all. The construction of a χ^2 distribution based on 700 simulations as in Fig. 1 would need a new generation of computers orders of magnitude faster than the present ones. Furthermore, the numerical computations were unstable when general models were fitted due to the large number of free parameters. This resulted in the paradox that the general model often seemed to be less probable than a special submodel.

Appendix 1

Sketch of the Proof of Theorem 1

The first rigorous proof of Theorem 1 was given by Billingsley (1961). Basawa and Rao (1980) gave a different kind of proof and, moreover, the theorem was proved self-containedly in Caliebe (1996).

The main idea of the χ^2 test is to compare the hypothesis with the general model: The maximum-likelihood transition probabilities (of the model) of the hypothesis are $p_{ij}(\hat{\phi})$, where $\hat{\phi}$ denotes the maximum-likelihood estimator of the parameter vector ϕ in the setup of the hypothesis (Eq. 3). The maximum-likelihood transition probabilities of the general model are n_{ij}/n_i (Billingsley, 1961; Basawa & Rao, 1980; Caliebe, 1996). Therefore, the expression $n_{ij}/n_i - p_{ij}(\hat{\phi})$ is a measure for the difference between the hypothesis and the general model. These differences are summed in the test statistic T_{th} (Eq. 5):

$$T_{th} = \sum_{\substack{i,j=1 \\ p_{i,j}(\phi)>0}}^{m} \frac{[n_{ij} - n_i p_{i,j}(\hat{\phi})]^2}{n_i p_{i,j}(\hat{\phi})} = \sum_{\substack{i,j=1 \\ p_{i,j}(\phi)>0}}^{m} \frac{[\frac{n_{ij}}{n_i} - p_{i,j}(\hat{\phi})]^2}{\frac{p_{i,j}(\hat{\phi})}{n_i}}. \quad \text{(A1.1)}$$

Theorem 1 states that T_{th} converges to a χ^2 distribution under the hypothesis. This corresponds to the intuition because in this case the differences between the models are small resulting in a small value of T_{th}. These small values fluctuate according to a χ^2 probability distribution. This distribution evolves because the differences are squared and properly normalized (here by $p_{ij}(\hat{\phi})/n_i$).

Since the proof of Theorem 1 is rather lengthy, only a sketch of the main arguments is given. For a detailed version *see* Caliebe (1996). We impose the following assumptions:

(i) Φ is an open interval of $\mathbb{R}^s$.
(ii) The functions $p_{i,j} : \Phi \to \mathbb{R}$, are twice continuously differentiable for all $i,j \in \{1, ..., m\}$.
(iii) The $m^2 \times s$ matrix $\left(\frac{\partial p_{i,j}}{\partial \phi_l}(\phi)\right)_{\substack{i,j\in\{1,\ldots,m\} \\ l\in\{1,\ldots,s\}}}$ has rank s for all $\phi \in \Phi$.

Let $P^0 = P(\phi^0)$ be the transition matrix which generates the data x with $\phi^0 \in \Phi$. Previous to proving the convergence of T_{th}, it is necessary to show the existence of the maximum-likelihood estimator $\hat{\phi}$. Therefore the likelihood equations Eq. 4 are derived and written in matrix notation. As an important feature, a rest term r appears. Then a sequence is recursively defined whose limiting value, if existent, satisfies the likelihood equations and is therefore the maximum-likelihood estimator. After estimating the value of the difference of r at two points, it is shown that the above sequence is a Cauchy sequence and thus converges.

Next the convergence of the maximum-likelihood estimator $\hat{\phi}$ to the real parameter value ϕ^0 is proved:

$$\sqrt{n}(\hat{\phi} - \phi^0) \xrightarrow{d}_n N_s(0, F^{-1}). \quad \text{(A1.2)}$$

(Here and in the following $N_k(a, M)$ is a k-dimensional normal distribution with mean a and covariance matrix M. For simplicity the covariance matrices are not specified in this sketch of the proof.)

This is done by using a Taylor approximation

$$\sqrt{n}(\hat{\phi} - \phi^0) = MX + \text{rest term} \quad \text{(A1.3)}$$

where M is a matrix. X is the following vector

$$X = \sqrt{n}(\hat{P}_V - P_V), \quad \text{(A1.4)}$$

with

$$P_V = (p^0_{1,1}, p^0_{1,2}, \ldots, p^0_{1,m}, \ldots, p^0_{m,1}, p^0_{m,2}, \ldots, p^0_{m,m})$$

$$\hat{P}_V = \left(\frac{n_{11}}{n_1}, \frac{n_{12}}{n_1}, \ldots, \frac{n_{1m}}{n_1}, \ldots, \frac{n_{m1}}{n_m}, \frac{n_{m2}}{n_m}, \ldots, \frac{n_{mm}}{n_m}\right)$$

being the vectors of the true transition probabilities of P^0 and of the maximum-likelihood probabilities in the general model.

It is known that

$$X \xrightarrow{d} N_{m^2}(0, C). \quad \text{(A1.5)}$$

By showing that the rest term in Eq. A1.3 vanishes one gets the desired, asymptotic distribution of Eq. A1.2 by a linear transformation of X.

After that the vector y is investigated whose components

$$y_{ij} := \frac{n_{ij} - n_i p_{ij}(\hat{\phi})}{\sqrt{p_{ij}(\hat{\phi}) n_i}}$$

are the individual (non-squared) terms of the sum of the test statistic T_{th}.

An evaluation in a Taylor series as in Eq. Al.3 results in

$$y \xrightarrow{d} N_{m^2}(0, D). \quad \text{(A1.6)}$$

If a random variable has a k-dimensional normal distribution it is a well known result that, under certain conditions, the sum of its squared components has a χ^2 distribution. Therefore

$$T_{th} = \sum_{\substack{i,j=1 \\ p_{i,j}(\phi)>0}}^{m} y_{ij}^2 \quad \text{(A1.7)}$$

is χ^2 distributed. The number of degrees of freedom of this distribution can be derived by calculating the eigenvalues of the covariance matrix D of Eq. A1.6.

Appendix 2

Calculation of $\hat{\phi}$ in the Two-Channel Model with Double Number of Channels

The likelihood equations Eq. 4

$$\frac{\partial L}{\partial \phi_l}(x, P(\phi)) = 0, \quad l = 1, 2, \ldots, s$$

can be written for a macro-channel with 4 parameters for the first parameter ϕ_l ($l = 1$)

$$\frac{\partial}{\partial \phi_1} \prod_{i,j=1}^{9} (p_{i,j}(\phi))^{n_{i,j}} = 0. \quad \text{(A2.1)}$$

Using the $p_{i,j}$ of Eq. 17 leads to the same functions of ϕ_1 with different n_{ij} as exponents in the product of Eq. A2.1. The n_{ij} related to the same basis are combined as follows

$$\begin{aligned}
N_1 &= 2n_{11} + n_{12} + 2n_{13} + n_{14} + 2n_{16} + n_{18} + n_{21} + n_{23} \\
&\quad + n_{26} + 2n_{31} + n_{32} + 2n_{33} + n_{34} + 2n_{36} + n_{38} + n_{41} \\
&\quad + n_{43} + n_{46} + 2n_{61} + n_{62} + 2n_{63} + n_{64} + 2n_{66} + n_{68} \\
&\quad + n_{81} + n_{83} + n_{86} \\
N_2 &= n_{12} + n_{14} + 2n_{15} + 2n_{17} + n_{18} + 2n_{19} + n_{25} + n_{27} \\
&\quad + n_{29} + n_{32} + n_{34} + 2n_{35} + 2n_{37} + n_{38} + 2n_{39} + n_{45} \\
&\quad + n_{47} + n_{49} + n_{62} + n_{64} + 2n_{65} + 2n_{67} + n_{68} + 2n_{69} \\
&\quad + n_{85} + n_{87} + n_{89} \\
N_3 &= n_{22} + n_{24} + n_{28} + n_{42} + n_{44} + n_{48} + n_{82} + n_{84} + n_{88}
\end{aligned} \quad \text{(A2.2)}$$

thus yielding

$$\frac{\partial}{\partial \phi_1}\left[(1-\phi_1)^{N_1} \phi_1^{N_2} (\phi_1\phi_2 + (1-\phi_1)(1-\phi_2))^{N_3}\right] = 0. \quad \text{(A2.3)}$$

Defining

$$M_1 = N_1 + N_2 + N_3, \quad M_2 = N_1 + 2N_2 + N_3,$$
$$M_3 = N_1 + 3N_2 + 2N_3 \quad \text{(A2.4)}$$

and calculating Eq. A2.3 results in

$$M_1\phi_1^2 - M_2\phi_1 + \phi_2(M_3\phi_1 - 2M_1\phi_1^2 - N_2) + N_2 = 0. \quad \text{(A2.5)}$$

In the case $l = 2$ it follows by the same procedure

$$K_1\phi_2^2 - K_2\phi_2 + \phi_1(K_3\phi_2 - 2K_1\phi_2^2 - L_2) + L_2 = 0 \quad \text{(A2.6)}$$

with

$$\begin{aligned}
L_1 &= n_{25} + n_{27} + n_{29} + n_{45} + n_{47} + n_{49} + n_{52} + n_{54} + 2n_{55} \\
&\quad + 2n_{57} + n_{58} + 2n_{59} + n_{72} + n_{74} + 2n_{75} + 2n_{77} + n_{78} \\
&\quad + 2n_{79} + n_{85} + n_{87} + n_{89} + n_{92} + n_{94} + 2n_{95} + 2n_{97} \\
&\quad + n_{98} + 2n_{99} \\
L_2 &= n_{21} + n_{23} + n_{26} + n_{41} + n_{43} + n_{46} + 2n_{51} + n_{52} + 2n_{53} \\
&\quad + n_{54} + 2n_{56} + n_{58} + 2n_{71} + n_{72} + 2n_{73} + n_{74} + 2n_{76} \\
&\quad + n_{78} + n_{81} + n_{83} + n_{86} + 2n_{91} + n_{92} + 2n_{93} + n_{94} \\
&\quad + 2n_{96} + n_{98} \\
L_3 &= N_3 \\
K_1 &= L_1 + L_2 + L_3, \quad K_2 = L_1 + 2L_2 + L_3, \\
K_3 &= L_1 + 3L_2 + 2L_3.
\end{aligned} \quad \text{(A2.7)}$$

Combining Eqs. A2.5 and A2.6 gives polynomials of order 5 in ϕ_1 resp. ϕ_2, which can be solved numerically.

When the rate constants (and therefore the transition probabilities) are small with respect to the sampling frequency of the data, the ϕ_l are near to 0. Then the following applies

$$\begin{aligned}
&N_2 \ll N_1, N_3, \quad N_2 \ll M_1, M_2, M_3, \quad M_1 \approx M_2 \approx M_3 \\
&L_2 \ll L_1, L_3, \quad L_2 \ll K_1, K_2, K_3, \quad K_1 \approx K_2 \approx K_3 \\
&2M_1\phi_1^2 \ll M_3\phi_1, \quad M_1\phi_1^2 \ll M_2\phi_1 \\
&2K_1\phi_2^2 \ll K_3\phi_2, \quad K_1\phi_2^2 \ll K_2\phi_2
\end{aligned} \quad \text{(A2.8)}$$

and yields an approximation of Eqs. A2.5 and A2.6:

$$\begin{aligned}
&-M_2\phi_1 + \phi_2(M_3\phi_1 - N_2) + N_2 = 0 \\
&-K_2\phi_2 + \phi_1(K_3\phi_2 - L_2) + L_2 = 0.
\end{aligned} \quad \text{(A2.9)}$$

$\hat{\phi}_2$ is the solution of a quadratic equation:

$$C_1\phi_2^2 + C_2\phi_2 + C_3 = 0 \quad \text{(A2.10)}$$

with

$$\begin{aligned}
C_1 &= M_3K_2 - N_2K_3 \\
C_2 &= N_2L_2 - M_3L_2 + N_2K_3 - M_2K_2 \\
C_3 &= -N_2L_2 + M_2L_2
\end{aligned} \quad \text{(A2.11)}$$

and $\hat{\phi}_1$ can be computed by

$$\hat{\phi}_1 = (-L_2 + K_2\hat{\phi}_2)/(K_3\hat{\phi}_2 - L_2). \quad \text{(A2.12)}$$

In the case of small rate constants, N_1 to N_3 and L_1, L_2 can be approximated by

$$\begin{aligned}
N_1 &= 2n_{11} + n_{12} + 2n_{13} + n_{21} + 2n_{31} + 2n_{33} + n_{34} \\
&\quad + 2n_{36} + n_{43} + 2n_{63} + 2n_{66} + n_{68} + n_{86} \\
N_2 &= n_{12} + n_{25} + n_{34} + n_{47} + n_{68} + n_{89} \\
N_3 &= n_{22} + n_{24} + n_{42} + n_{44} + n_{48} + n_{84} + n_{88} \\
L_1 &= n_{25} + n_{47} + n_{52} + 2n_{55} + 2n_{57} + n_{74} + 2n_{75} + 2n_{77} \\
&\quad + 2n_{79} + n_{89} + 2n_{97} + n_{98} + 2n_{99} \\
L_2 &= n_{21} + n_{43} + n_{52} + n_{74} + n_{86} + n_{98}.
\end{aligned} \quad \text{(A2.13)}$$

$\hat{\phi}_3$ and $\hat{\phi}_4$ can be calculated analogously.

Appendix 3

Calculation of the Probabilities of False Jumps

As explained above (*see* Application to Noisy Data), the failure of the test in the presence of high noise originates from incorrectly identified transitions n_{23} and n_{32}. They result mainly from "false jumps": A jump takes place and is detected, but due to the superimposed noise the target state of the jump is falsely identified. Usually, if the dwell-time of the new state is long enough, the detector can realize the error and reports a second jump shortly after the false jump, now to the correct target state. This is illustrated by the following example: The Markov chain produces a transition from state 1 to state 3. The detector first registers a transition from state 1 to state 2 and then a transition from state 2 to state 3. Thus, these false transitions increase n_{23}. The following calculations give an estimation of the expected number of the falsely identified jumps n_{23} and their standard deviation. In the case of n_{32} similar considerations apply. The calculations consist of the expected number of falsely identified jumps n_{12} and are based on the following recursive calculation of the test values $h_{k,t}$ of two parallel operating Sublevel-Hinkley detectors (Draber & Schultze, 1994) aiming at the current levels i_2 of state 2 and i_3 of state 3:

$$h_{k,t} = \max(h_{k,t-1} + p_k(e_t - p_k), 0) \qquad k = 2,3 \tag{A3.1}$$

with t being the sampling time, p_2 and p_3 the half jump amplitudes between the current level i_1 of state 1 and the current levels i_2 or i_3, respectively, and $e_t = i(t) - i_1$ being the difference between the sampled current value $i(t)$ and i_1 of state 1. If the test value $h_{k,t}$ of the Hinkley detector for state k crosses a preset threshold ϵ, a jump to this state is detected. The starting value for $h_{k,t}$ after a jump is detected, is zero. Because of the parabolic dependence on p_k in Eq. A3.1, the test value aiming at the correct current level usually crosses the threshold first (Draber & Schultze, 1994). $\in$ is adjusted to the magnitude of the noise:

$$\epsilon = 8\sigma^2. \tag{A3.2}$$

After a jump from state 1 to state 3 at $t = 0$ and in the absence of a second jump the test values $h_{k,t}$ increase monotonously with high probability so that one gets recursively

$$h_{k,t} = p_k\left(\sum_{m=0}^{t} e_m - (t+1)p_k\right). \tag{A3.3}$$

Replacing $\sum_{m=0}^{t} e_m$ for h_2, t leads to

$$h_{3,t} = \frac{p_3}{p_2} h_{2,t} + (t+1)p_3(p_2 - p_3). \tag{A3.4}$$

In the case of white noise (with standard deviation σ), $h_{k,t}$ is normally distributed with mean $(t+1)i_3\, p_k - (t+1)p_k^2$ and standard deviation $(t+1)p_k^2\sigma^2$ (Eq. A3.3).

Two different cases have to be considered that result in detecting a jump from 1 to 2 instead of from 1 to 3:

A: $h_{2,t}$ crosses the threshold $\in$ earlier than $h_{3,t}$.

B: $h_{2,t}$ and $h_{3,t}$ cross $\in$ at the same time, but $h_{2,t} > h_{3,t}$.

If the next jump takes place at time t_0, the probability for case A is (P_3 is the probability given a jump from 1 to 3)

$$\begin{aligned} P_A := & P_3(\min\{t \in \{0,\ldots,t_0\} : h_{2,t} > \epsilon\} \\ & < \min\{t \in \{0,\ldots,t_0\} : h_{3,t} > \epsilon\}) \\ & = \sum_{n=0}^{t_0} P_3(h_{2,n} > \epsilon, h_{2,n-1} < \epsilon, h_{3,n} < \epsilon). \end{aligned} \tag{A3.5}$$

Introducing Eqs. A3.1 and A3.4 leads to

$$\begin{aligned} P_A = \sum_{n=0}^{t_0} & P_3(h_{2,n-1} + p_2(e_n - p_2) > \epsilon, h_{2,n-1} < \epsilon, \\ & \frac{p_3}{p_2} h_{2,n} + (n+1)p_3(p_2 - p_3) < \epsilon). \end{aligned} \tag{A3.6}$$

$h_{2,n}$ is replaced by $h_{2,n-1}$ (Eq. A3.1):

$$\begin{aligned} P_A = \sum_{n=0}^{t_0} & P_3(h_{2,n-1} + p_2(e_n - p_2) > \epsilon, h_{2,n-1} < \epsilon, \\ & \frac{p_3}{p_2} h_{2,n-1} + p_3(e_n - p_2) + (n+1)p_3(p_2 - p_3) < \epsilon) \\ = \sum_{n=0}^{t_0} & P_3(h_{2,n-1} < \epsilon, \\ & \frac{\epsilon}{p_3} - \frac{h_{2,n-1}}{p_2} - (n+1)(p_2 - p_3) + p_2 > e_n \\ & > \frac{\epsilon - h_{2,n-1}}{p_2} + p_2). \end{aligned} \tag{A3.7}$$

Let f_1 be the density of e_n and f_2 the density of $h_{2,n-1}$. Since they are densities of normal distributions, the probability that $h_{2,t}$ crosses first the threshold ϵ can be thus obtained by numerical integration:

$$P_A = \sum_{n=0}^{t_0} \int_0^{\epsilon} \int_{y_1}^{y_2} f_1(x) f_2(y)\, dx\, dy \tag{A3.8}$$

with $y_1 = \frac{\epsilon - x}{p_2} + p_2$ and $y_2 = \frac{\epsilon}{p_3} - \frac{x}{p_2} - (n+1)(p_2 - p_3) + p_2$.

P_B can be evaluated in a similar way:

$$P_B = \sum_{n=0}^{t_0} \int_0^{\min(\epsilon, \frac{p_2}{p_3}(\epsilon - np_3(p_2 - p_3)))} \int_{y_3}^{y_4} f_1(x) f_2(y)\, dx\, dy \tag{A3.9}$$

with $y_3 = \max(\frac{\epsilon - x}{p_2} + p_2, \frac{1}{p_3}(\epsilon(n+1)p_3(p_2 - p_3)) - \frac{x}{p_2} + p_2)$ and $y_4 = (n+1)p_3 - \frac{x}{p_2} + p_2$.

The theoretical expectation and standard deviation of falsely identified transitions n_{23}^{th} from 2 to 3 originating in a jump from 1 to 3 can be calculated from P_A and P_B.

$$\begin{aligned} & E(n_{23}^{\text{th}}) = n_{13}(P_A + P_B) \text{ and} \\ & \sigma(n_{23}^{th}) = \sqrt{n_{13}(P_A + P_B)(1 - (P_A + P_B))}. \end{aligned} \tag{A3.10}$$

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