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Coupled and uncoupled Markov systems: a possible way to distinguish between them

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Abstract. The conditional distributions of openings and closings are computed for Markov schemes with two open and two closed states and with different pathways connecting the open and closed aggregates. The computation is performed for uncoupled schemes by directly applying the probability laws and by using a convolution algorithm for coupled schemes. The results show that, for coupled schemes, conditional distributions can be non-monotonic functions of the dwell time duration. Simulations, illustrating how the difference between coupled and uncoupled models can be detected, are also reported.

Key words: Ion channels – Coupled Markov systems – Conditional distributions

Introduction

Patch-clamp recording from a single ion channel makes it possible to observe directly the transitions between conducting and non-conducting states of the channel.

Much theoretical work has been done to obtain an interpretation of the signals recorded in patch-clamp (Colquhoun and Hawkes 1981, 1983, 1987; Fredkin et al. 1985; Kirber et al. 1985; Kienker 1989). A simple interpretation is that each open or closed state is left with a constant probability per unit time. The corresponding kinetic scheme is thus a Markov scheme with discrete states.

However, what are observed in a patch-clamp recording are not the transitions between single states, but rather the transitions between the aggregate of open states and closed states. Simple inspection of the recording does not say in which state the system is but only in which aggregate it is. Thus, when several states with the same conductance are connected, transitions can occur which cannot be directly detected in the recorded signal. The existence of such "hidden" transitions makes it very difficult to reconstruct the underlying kinetic scheme from the analysis of the recording.

Markov systems where hidden transitions can occur are called "coupled", whereas those where such transitions can not occur are "uncoupled" (Kienker 1989). It is clear that in uncoupled Markov systems the distribution of openings and that of closings are given by linear combinations of decreasing exponentials. In Scheme (1 a), below, for example, the dwell times at the conductive level (open channel) belong to the subset of O_1 openings or to the subset of O_2 openings and the probability per unit time of leaving O_1 or O_2 is constant.

It can be shown (Kirber et al. 1985) that the same conclusion (decomposition into exponential components of the dwell time distributions) holds for coupled systems satisfying the detailed balance.

What can be deduced from a patch clamp recording about the system which generates the recorded sequence of openings and closings? Finding a Markov scheme which accounts for the data is still an open problem.

Statistical methods have been used to test the fit of a model to the experimental data; for instance the maximum likelihood method has been used either to estimate the number of channels in a patch or to rank models fitted to the recorded sequence of openings and closings (Horn and Lange 1983; Ball and Sansom 1989). The alternative method proposed here allows different classes of models to be discriminated, on the basis of the qualitative features of the activity patterns they generate.

A serious difficulty arises when one tries to reconstruct the kinetic model from experimental data: the finite bandwidth of the recording system causes the loss of the short events (for instance of the short closings) and so the lumping of consecutive openings together.

In spite of this, it is relatively easy to distinguish between different classes of models. For instance the sequences produced from models such as Scheme (1 a) and (1 b) are clearly different. In the sequences generated from Scheme (1 b) there cannot be any correlation between consecutive intervals; while in the sequences from Scheme (1 a) a correlation can be found, because different gateway

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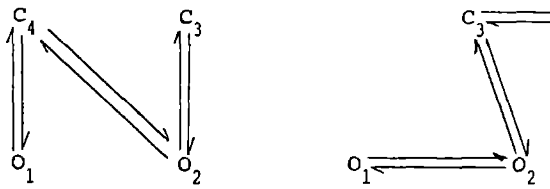
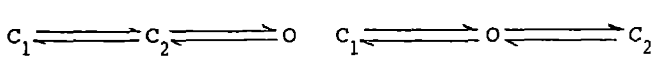
states connect the open to the closed aggregate (Fredkin et al. 1985).



Scheme 1 a **b**

But the next step, i.e. the complete reconstruction of the kinetic scheme is very difficult, even if the specific problem involved in analysing experimental data is bypassed by confining the analysis to simulated data (or to the analytical formulation of the problem), as we will do here.

We will consider particular cases and try to find out what conclusions can be drawn about the systems which generate the data. In particular, is there only one system which can generate the considered sequence of openings and closings? In general, the answer is negative. For instance, Schemes (2 a) and (2 b) below can yield sequences



Scheme 2 a **b**

which are statistically identical. Both schemes generate sequences without correlation and it is clear that, assigning suitable rate constants, Scheme (2 b) can generate any required combination of exponential components.

The same conclusion can be drawn by using similarity transformations on the matrix representation of a kinetic scheme to obtain the equivalent model (two models are called equivalent when they have the same observable steady state statistics). This is the method used by Kienker (1989). It is a very general method; the equivalence of very complex models can be examined even in cases when intuition is not very helpful. A general result obtained by using similarity transformations is that, formally, every Markov system with discrete states is equivalent to a reduced uncoupled scheme with the same number of open and closed states.

In the present work it is shown, for a particular case, that this equivalence holds only formally. We will consider a coupled scheme with two closed and two open states, which generates correlation in the sequence of dwell times, and we will show that in this case the equivalent uncoupled scheme should have negative rate constants, which have no physical meaning. The result can be generalized to any model with a chain of closed (open) states connecting two open (closed) states. On the other hand, the need to check the result of the similarity transformation is clearly stated in Kienker's paper.

We will reach these results by computing the conditional distribution of dwell times in the two aggregates of closed and open states; however this approach is equivalent to that of the similarity transformations.

Computation of the conditional distributions could have been omitted, as their expression, obtained in a general form by the matrix method, has already been reported by Fredkin et al. (1985) and subsequently by Steinberg (1987) and Bauer et al. (1987). Nevertheless, we will perform the computation ex-novo for the particular kinetic schemes we consider, because the approach used illustrates better the probabilistic meaning of the terms which appear in the final equations.

Examination of the analytical expressions obtained will enable us to show how the links closed-closed or open-open can be detected by using conditional distributions. Therefore, links between states with the same conductance cannot a priori be excluded from models fitted to experimental data, contrary to what other people have written (Bauer et al. 1987). Thus, the conditional distributions are useful not only to identify the kinetic models, but also as a tool of theoretical investigation.

To illustrate how the signs of theoretical transitions can be detected, sequences of openings and closings generated by simple kinetic models will be analzyed.

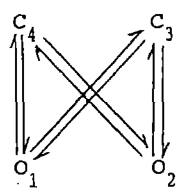
Section 1 – uncoupled models

Let us firstly consider uncoupled models with two closed and two open states. We call n_i the number of exits from state i in a hypothetical very long record, and Θ_i $(i=1, 2)$ the time constants for the openings and τ_i $(i=3, 4)$ those for the closings.

For the most general model of this kind (scheme 3 below) the pair of closed and open times distributions is completely characterized by six parameters, the four time constants and two weights, one for openings and one for closings; in contrast, in the model there are eight unknown rate constants, that will hereafter be called k_{ij}.

As shown in Appendix I, even a simplified model (N-model) with only six non-zero rate constants can account for any pair of distributions with arbitrarily chosen time constants and weights. Instead, the use of conditional distributions makes it possible to determine all the rate constants of uncoupled schemes (Fredkin et al. 1985; Kienker 1989).

To prove that, let us compute for the model of scheme (3) the probability density function (pdf) $f_{C,O}(t_c, t_o)$ of an



Scheme 3

opening-closing pair of durations t_o and t_c respectively. This calculation is considerably simpler here than for coupled schemes, since each state is characterized by its time constant, which can easily be derived from the kinetic scheme; for instance for state O_1 we get $\Theta_1 = (k_{14} + k_{13})^{-1}$, for state C_3 we get $\tau_3 = (k_{31} + k_{32})^{-1}$, and so on.

Let P_{ij} be the probability that the channel next enters state j after leaving state i. Then we have:

$$P_{23} = k_{23}/(k_{23} + k_{24}) \quad P_{24} = k_{24}/(k_{23} + k_{24}) \tag{1.1}$$
$$P_{13} = k_{13}/(k_{13} + k_{14}) \quad P_{14} = k_{14}/(k_{13} + k_{14})$$

Moreover let us define:

$$N_1 = n_1/(n_1+n_2) \tag{1.2}$$
$$N_2 = n_2/(n_1+n_2)$$

In the experimental case N_1 or N_2 ($N_1+N_2=1$) can be directly estimated as the weights of the two components of openings fitted to the global distributions.

Finally, to have more compact expressions, we introduce the notation $\Phi_\sigma(t)=1/\sigma \exp(-t/\sigma)$ for the normalized exponential distribution with time constant σ.

Then we have:

$$f_{c,o}(t_c, t_o) = N_1\, \Phi_{\theta_1}(t_o)[P_{14}\, \Phi_{\tau_4}(t_c) + P_{13}\, \Phi_{\tau_3}(t_c)] + N_2\, \Phi_{\theta_2}(t_o)[P_{24}\, \Phi_{\tau_4}(t_c) + P_{23}\, \Phi_{\tau_3}(t_c)] \tag{1.3}$$

For the analysis of experimental (simulated) data it is worth using, instead of $f_{c,o}(t_c, t_o)$, an integral of it; for instance, the distribution $\Omega(t/X)$ of closed intervals preceded by openings longer than X, which is obtained by integrating (1.3) from X to infinity:

$$\Omega(t, X) = [N_1\, P_{14} \exp(-X/\Theta_1) + N_2\, P_{24} \exp(-X/\Theta_2)] \cdot \Phi_{\tau_4}(t) + [N_1\, P_{13} \exp(-X/\Theta_1) + N_2\, P_{23} \exp(-X/\Theta_2)]\, \Phi_{\tau_3}(t) \tag{1.4}$$

This is a distribution of closed intervals, where the weights of the τ_3 and τ_4 components depend on X.

Likewise, by calling N_3 and N_4 the weights of the close components, the p.d.f. $\Gamma(t/X)$ of openings lasting for t preceded by closings longer than X can be written as:

$$\Gamma(t/X) = [N_3\, P_{32} \exp(-X/\tau_3) + N_4\, P_{42} \exp(-X/\tau_4)] \cdot \Phi_{\theta_2}(t) + [N_3\, P_{31} \exp(-X/\tau_3) + N_4\, P_{41} \exp(-X/\tau_4)]\, \Phi_{\theta_1}(t) \tag{1.5}$$

where:

$$P_{42} = k_{42}/(k_{41}+k_{42}) \quad P_{41} = k_{41}/(k_{41}+k_{42}) \tag{1.6}$$
$$P_{32} = k_{32}/(k_{32}+k_{31}) \quad P_{31} = k_{31}/(k_{32}+k_{31})$$

and:

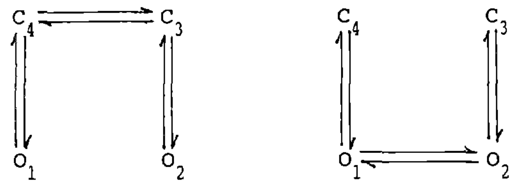
$$N_3 = n_3/(n_3+n_4) \quad N_4 = n_4/(n_3+n_4) \tag{1.7}$$

The expression for $\Omega(t/X)$ and $\Gamma(t/X)$ can be described intuitively. For instance, in (1.5), the term $N_3 \exp(-X/\tau_3)$ gives the percentage of closings from state C_3 that are longer than X. Multiplication by P_{32} gives the fraction of these closings going to open state O_2. So the coefficients of $\Phi_{\theta_1}(t)$ and $\Phi_{\theta_2}(t)$ in (1.5) are the sums of the percentages of transitions from states C_3, C_4 towards states O_1 and O_2 under the applied condition. This intuitive way of reading (1.5) and (1.6) enables us to claim that for any uncoupled model (with an arbitrary number of states) each coefficient of coupling between exponential components of openings and closings is non-negative. This point is relevant for the comparison with coupled models.

We note that expressions (1.4) and (1.5) can be used to completely determine the parameters of uncoupled models (Fredkin et al. 1985; Petracchi and Barbi 1991). In fact, N_1 and N_3 (and their complements to unity N_2 and N_4) are the weights of the components in the global distributions, and they can be measured. Then the conditional distributions allow the P_{ij} values to be determined, and combining the expressions for the time constants and (1.6) the values of the rate constants can be computed.

Section 2 – coupled models

Now let us consider the π-shaped models shown below: is it possible to discriminate these schemes, where transitions between states with the same conductance can occur, from uncoupled schemes?



Scheme 4a **b**

As shown in Appendix I, uncoupled models can reproduce any global distributions of openings and closings, and therefore also those generated by a π-shaped model. To investigate the discriminating capability of the conditional distributions, we will derive their analytical expression for Scheme (4a) above.

But firstly we stress that when dealing with coupled models a clear distinction must be made between dwell times in closed or open states (which are exponentially distributed) and closed or open intervals. In this section we will always consider dwell times in the closed aggregate, and not dwell times in a single closed state.

For scheme (4a), the time constants Θ_1 and Θ_2 of openings are simply the reciprocals of the rate constants k_{14} and k_{23}. The time constants of closings are harder to obtain since, from closed state C_3, the channel can go to closed state C_4, come back to C_3 and repeat such transitions many times before entering the open states O_2 or O_1.

The method commonly used to get the distributions of closings and openings (Colquhoun and Hawkes 1983) involves writing and solving a system of differential equations for the functions $P_{ij}^*(t)$, defined as the probabilities that the system is in state i at time zero and exits to state j at time t, without ever previously leaving the aggregate to which state i belongs.

We will use a slightly different approach, which uses the calculation of convolution integrals to get the functions $f_{31}(t)$, $f_{32}(t)$, $f_{41}(t)$, $f_{42}(t)$, namely the probabilities of channel being in state C_3 or C_4 at time 0 and opening to state O_1 or O_2 at time t, without ever previously opening.

Suppose the system is in state C_4 at time 0; the probability density $f_{41}(t)$ of it exiting to state O_1 at time t without ever opening in the interval (0, t) is the sum of two terms. The first accounts for the probability of a direct transition from state C_4 to state O_1 at time t and therefore is a normalized exponential with time constant $\tau_4 = (k_{41}+k_{43})^{-1}$ multiplied by the probability $k_{41}/(k_{41}+k_{43})$ of this way out from C_4; the second one represents the probability that the system leaves C_4 to enter C_3

at time x ($x<t$) and then, after possibly going and coming between states C_3 and C_4, opens to state O_1 at time t. Thus we get the expression:

$$f_{41}(t)=[k_{41}/(k_{41}+k_{43})]\,\Phi_{\tau_4}(t)+[k_{43}/(k_{41}+k_{43})]\int_0^t \Phi_{\tau_4}(x) f_{31}(t-x)\,dx \quad (2.1)$$

Likewise it follows that:

$$f_{31}(t)=[k_{34}/(k_{32}+k_{34})]\int_0^t \Phi_{\tau_3}(x)\, f_{41}(t-x)\,dx \quad (2.2)$$

Laplace transformation of the last two equations yields the system of equations:

$$f_{41}^*(s)=[k_{41}/(k_{41}+k_{43})]\,\Phi_{\tau_4}^*(s)+[k_{43}/(k_{41}+k_{43})]\cdot\Phi_{\tau_4}^*(s)\, f_{31}^*(s) \quad (2.3)$$

$$f_{31}^*(s)=[k_{34}/(k_{32}+k_{34})]\,\Phi_{\tau_3}^*(s)\, f_{41}^*(s) \quad (2.4)$$

whence, by eliminating $f_{31}^*(s)$ and with simple algebra:

$$f_{41}^*(s)=\frac{[k_{41}/(k_{41}+k_{43})]\,\Phi_{\tau_4}^*(s)}{1-[k_{43}\,k_{34}/(k_{41}+k_{43})\,(k_{34}+k_{32})]\,\Phi_{\tau_4}^*(s)\,\Phi_{\tau_3}^*(s)} \quad (2.5)$$

Further, by remembering that $\Phi_\sigma^*=\sigma^{-1}/(\sigma^{-1}+s)$, we have the following explicit expression:

$$f_{41}^*(s)=\frac{k_{41}/(s+k_{41}+k_{43})}{1-k_{43}\,k_{34}/[(s+k_{41}+k_{43})\,(s+k_{34}+k_{32})]}=\frac{k_{41}(s+k_{34}+k_{32})}{(s-\alpha)\,(s-\beta)} \quad (2.6)$$

where α and β are the solutions of the quadratic equation:

$$s^2+(k_{41}+k_{43}+k_{34}+k_{32})\,s+k_{41}\,k_{34}+k_{41}\,k_{32}+k_{43}\,k_{32}=0 \quad (2.7)$$

Since $\Delta=(k_{41}+k_{43}-k_{34}-k_{32})^2+4k_{43}\,k_{34}>0$, α and β are real and negative for every choice of the rate constants.

Finally, by taking the inverse Laplace transform, we get the distribution of closings starting in state C_4 and ending in state O_1:

$$f_{41}(t)=\frac{k_{41}}{\alpha-\beta}\cdot[(k_{32}+k_{34}+\alpha)\exp(\alpha t)-(k_{32}+k_{34}+\beta)\exp(\beta t)] \quad (2.8)$$

Analogously, we get for $f_{31}(t)$:

$$f_{31}(t)=k_{41}\,k_{34}(\exp(\alpha t)-\exp(\beta t))/(\alpha-\beta) \quad (2.9)$$

A similar calculation yields the distributions of closings terminating in state O_2 as:

$$f_{32}(t)=\frac{k_{32}}{\alpha-\beta}\cdot[(k_{41}+k_{43}+\alpha)\exp(\alpha t)-(k_{41}+k_{43}+\beta)\exp(\beta t)] \quad (2.10)$$

and:

$$f_{42}(t)=k_{32}\,k_{43}(\exp(\alpha t)-\exp(\beta t))/(\alpha-\beta) \quad (2.11)$$

Functions $f_{32}(t)$, $f_{31}(t)$, $f_{42}(t)$, $f_{41}(t)$ allow us to write every distribution of closings. For instance the probability densities $f_3(t)$ and $f_4(t)$ of channel opening at time t, having been in state C_3 or C_4 respectively at time 0, are given by:

$$f_3(t)=f_{31}(t)+f_{32}(t),\quad f_4(t)=f_{41}(t)+f_{42}(t) \quad (2.12)$$

The functions $f_3(t)$ and $f_4(t)$ are the probability densities of the subsets of closed intervals which begin in closed states 3 or 4 respectively. They are conditional distributions and do not coincide with the distributions of dwell times in C_3 and C_4 (which are obviously single exponentials). Note that $f_3(t)$ and $f_4(t)$ are algebraic sums of two exponential components, i.e. the closings starting in each closed state are characterized by two time constants. Figure 1 shows the distributions $f_{31}(t)$, $f_{32}(t)$ and $f_3(t)$ for the π-shaped model with the parameters given in the caption. Unlike $f_{32}(t)$, which is monotonically decreasing, $f_{31}(t)$ presents a sharp maximum. The function $f_3(t)$ may or may not have a maximum depending on the values of parameters (see Appendix III). But functions $f_{31}(t)$ and $f_3(t)$ cannot be determined experimentally, as it is not possible to assess in which state of the closed aggregate the system is at any moment.

Let us compute now the conditional distributions which are really of interest, obtained by imposing conditions on the previous interval. The bidimensional distribution of openings of duration t_o followed by closings of duration t_c is given by:

$$f_{C,O}(t_c,t_o)=N_2\,\Phi_{\theta_2}(t_o)\,f_3(t_c)+N_1\,\Phi_{\theta_1}(t_o)\,f_4(t_c) \quad (2.13)$$

Here N_1 and N_2 are, as in Sect. 1, the percentages of openings from state O_1 or from state O_2. From (2.13), by integration over t_o from X to infinity, the distribution $\Omega(t/X)$ of closings preceded by openings longer than X follows:

$$\Omega(t/X)=N_2\exp(-k_{23}\,X)\,f_3(t)+N_1\exp(-k_{14}X)\,f_4(t) \quad (2.14)$$

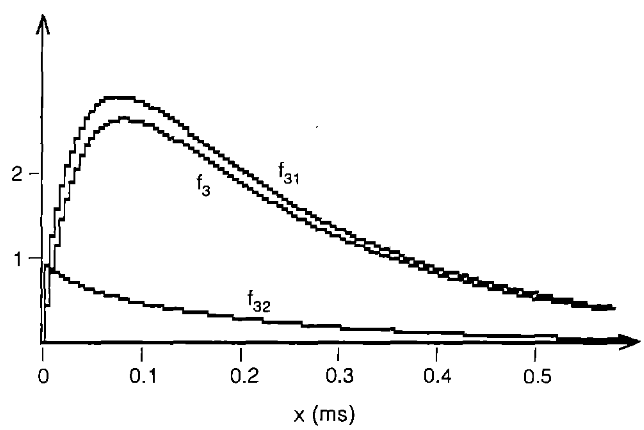


Fig. 1. Plot of functions $f_{31}(t)$, $f_{32}(t)$ and $f_3(t)$ computed in the text for the π-scheme; the function $f_{32}(t)$ is magnified by a factor 2. The rate constants in conventional m s^{-1} units are: $k_{14}=10$, $k_{41}=10$, $k_{23}=0.1$, $k_{32}=0.5$, $k_{43}=10$, $k_{34}=10$. According to the results of Appendix III $f_3(t)$ is a non-monotonic function

Analogously, for the distribution of openings of duration t_0 following the closings of duration t_c we can write:

$$f_{o,C}(t_o, t_c) = [N_2\, f_{32}(t_c) + N_1\, f_{42}(t_c)]\Phi_{\theta_2}(t_0) + [N_2\, f_{31}(t_c) + N_1\, f_{41}(t_c)]\Phi_{\theta_1}(t_0) \quad (2.15)$$

In fact, the percentage of closings starting in state C_3 (C_4) is equal to the percentage of openings in $O_2(O_1)$ and so is given by $N_2(N_1)$ (see Appendix II). Note that N_3 and N_4 are different from N_1 and N_2 respectively and their use in (2.15) would be wrong.

By integrating (2.15) over t_c from X to infinity:

$$\Gamma(t/X) = \Phi_{\theta_2}(t) \int_X^\infty [N_2\, f_{32}(t_c) + N_1\, f_{42}(t_c)]\, dt_c + \Phi_{\theta_1}(t) \int_X^\infty [N_2\, f_{31}(t_c) + N_1\, f_{41}(t_c)]\, dt_c \quad (2.16)$$

By using the notation $\Phi_\sigma(t) = 1/\sigma \exp(-t/\sigma)$ and $\Psi_\delta(t) = |\delta| \exp(\delta t)$ for the normalized exponential distributions, (2.14) and (2.16) can be written in the final form:

$$\Omega(t/X) = [Z_{\alpha,1} \exp(-k_{14} X) + Z_{\alpha,2} \exp(-k_{23}\, X)]\, \Psi_\alpha(t) + [Z_{\beta,1} \exp(-k_{14} X) + Z_{\beta,2} \exp(-k_{23}\, X)]\, \Psi_\beta(t) \quad (2.17)$$

and

$$\Gamma(t/X) = [Z_{\alpha,1} \exp(-\alpha X) + Z_{\beta,1} \exp(-\beta X)]\, \Phi_{\theta_1}(t) + [Z_{\alpha,2} \exp(-\alpha X) + Z_{\beta,2} \exp(-\beta X)]\, \Phi_{\theta_2}(t) \quad (2.18)$$

where:

$$Z_{\alpha,1} = \frac{k_{41} k_{34}}{k_{32} k_{43} + k_{41} k_{34}} \frac{|\beta| - k_{41}}{\alpha - \beta} \quad (2.19)$$

$$Z_{\alpha,2} = \frac{k_{32} k_{43}}{k_{32} k_{43} + k_{41} k_{34}} \frac{|\beta| - k_{32}}{\alpha - \beta}$$

$$Z_{\beta,1} = \frac{k_{41} k_{34}}{k_{32} k_{43} + k_{41} k_{34}} \frac{k_{41} - |\alpha|}{\alpha - \beta}$$

$$Z_{\beta,2} = \frac{k_{32} k_{43}}{k_{32} k_{43} + k_{41} k_{34}} \frac{k_{32} - |\alpha|}{\alpha - \beta}$$

Now we note that the Z-coefficients cannot be all positive, a feature not shared by uncoupled models (see (1.4) and (1.5)). We will show that one of the Z-coefficients is always negative. To obtain a proof, one only needs to consider the sign of the product $Z_{\alpha,1} \times Z_{\alpha,2}$, which can be written:

$$\text{Sign}(Z_{\alpha,1}\, Z_{\alpha,2}) = -\text{Sign}[\alpha\beta + k_{32}(\alpha + \beta) + k_{32}\, k_{32}] \quad (2.20)$$

and by substituting to $\alpha\beta$ and $\alpha + \beta$ their expressions from (2.7):

$$\text{Sign}(Z_{\alpha,1}\, Z_{\alpha,2}) = \text{Sign}(k_{32} - k_{41}) \quad (2.21)$$

By the same procedure we get:

$$\text{Sign}(Z_{\beta,1}\, Z_{\beta,2}) = -\text{Sign}(k_{32} - k_{41}) \quad (2.22)$$

In conclusion, coefficients $Z_{\alpha,j}$ and $Z_{\beta,j}$ cannot all have the same sign. Given their meaning, three of them must be positive and one negative; and, since the distributions have to be always positive, the negative coefficient must be related to the components with smaller time constants.

The negative sign of one of the Z-coefficients shows that the equivalence between the coupled π-scheme and an uncoupled scheme only holds formally. One way to find the uncoupled scheme formally equivalent to the π-scheme is to determine the Z-coefficients, what in the experimental case can be done by fitting (2.17) or (2.18) to the corresponding conditional distributions. The transition rates of the uncoupled model are then computed following the line given at the end of Sect. 1. So it becomes clear that from a negative Z-coefficient a negative transition rate is obtained.

A different approach based on using the similarity transformations (Kienker 1989) could be used, but it should produce the same results. In fact, the uncoupled model obtained from a coupled one through a similarity transformation is characterized by the same observable statistics, namely the same global and conditional distributions (Fredkin et al. 1985).

The occurrence of a negative Z-coefficient is a qualitative difference between π-shaped and uncoupled models. Even if numerically smaller than the other coefficients which appear in (2.18), a negative Z-coefficient can be detected by analysing the data. In particular, if the components of openings are well separated, it is possible to obtain conditional distributions which are algebraic sums of exponentials and which exhibit a maximum.

Let us consider again the numerical case of Fig. 1, where the transition rates are given in conventional units called ms^{-1}; the components of the openings are, in this numerical case, well separated, having time constants $\Theta_1 = 0.1$ ms and $\Theta_2 = 10$ ms. Hereafter, as in Appendix III, we call α the greater of the two solutions of the characteristic equation (2.7) and β the smaller ($\alpha = -4.18$, $\beta = -26.32$). Then expressions (2.19) give:

$$Z_{\alpha,1} = 0.70, \quad Z_{\alpha,2} = 0.055, \quad Z_{\beta,1} = 0.25, \quad Z_{\beta,2} = -0.0079, \quad (2.23)$$

and the probability density of a closing of duration t preceded by an opening longer than X, can be written as:

$$\Omega(t/X) = [0.25 \exp(-X/0.1) - 0.0079 \exp(-X/10)]\, \psi_\beta(t) + [0.7 \exp(-X/0.1) - 0.055 \exp(-X/10)]\, \psi_\alpha(t) \quad (2.24)$$

When X is large enough, the terms with the shortest exponential can be neglected and (2.24) can be written:

$$\Omega(t/X) = -0.0079 \exp(-X/10)\, \psi_\beta(t) + 0.055 \exp(-X/10)\, \psi_\alpha(t) \quad (2.25)$$

and numerically, for $X = 1$ ms:

$$\Omega(t/X) = -0.0079 \exp(-0.1)\, \psi_\beta(t) + [0.055 \exp(-0.1)]\, \psi_\alpha(t) \quad (2.26)$$

This function, which can be determined experimentally, is plotted in Fig. 2; it looks very similar to function $f_3(t)$ plotted in Fig. 1 and like $f_3(t)$ has a clear maximum. In Fig. 3 we report the conditional histogram obtained by simulating the π-scheme with the parameters of Fig. 1 and Fig. 2.

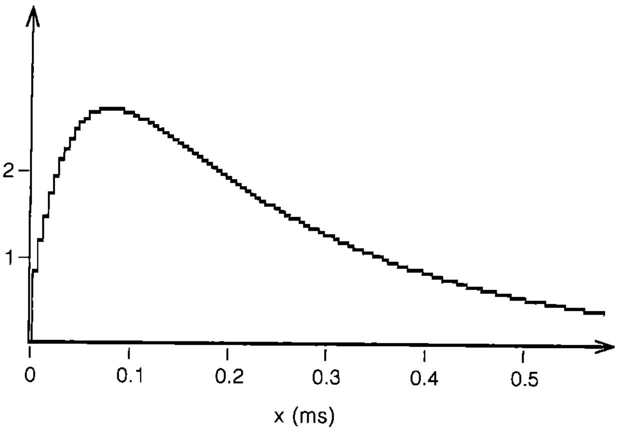


Fig. 2. Conditional distribution of closings preceded by openings longer than 1 ms (conventional unit) computed for the same numerical case as Fig. 1

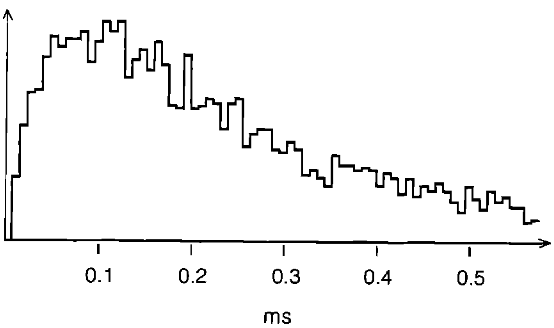


Fig. 3. Conditional distribution of closings preceded by openings longer than 1 ms, obtained by simulating the π-scheme with the numerical values given in the caption to Fig. 1. 5200 openings out of a total number of 120 000 were selected by the condition. Dwell times are reported on a linear scale

The possibility of obtaining non-monotonic conditional distributions is typical of coupled schemes. On the other hand the global distributions are always monotonic functions. The global distribution of closings is given in our case by $f(t) = N_2\, f_3(t) + N_1\, f_4(t)$, and from (2.16):

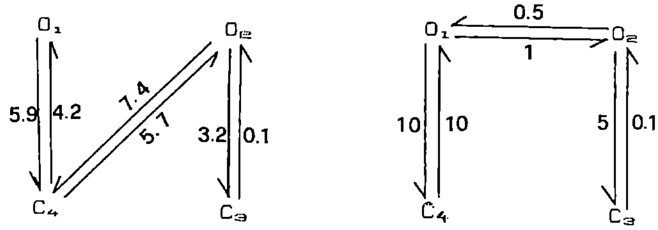
$$f(t) = [Z_{\alpha,1} + Z_{\alpha,2}]\, \psi_\alpha(t) + [Z_{\beta,1} + Z_{\beta,2}]\, \psi_\beta(t) \qquad (2.27)$$

In (2.27) the coefficients of both exponential components are positive. Therefore, only by using conditional distributions, the difference between the coupled π-scheme and the uncoupled schemes can be detected.

The difference between a π-scheme and schemes without hidden transitions can also be detected in more general cases, namely when the conditional distributions are monotonic functions. Consider the π-scheme reported below: for the parameters quoted, the conditional distribution of closings is monotonically decreasing (see Appendix III), but a difference from uncoupled models can still be detected in the simulated data.

As a first step, we determine which of the schemes without hidden transitions is the closest to the π-scheme. An N-scheme which replaces the negative coefficient which appears in (2.17) and (2.18) with zero is the obvious answer to this requirement. Then, by using the expressions in Appendix I, we can obtain the rate constants of the N-scheme which produces the same global distributions as the π-scheme and gives the approximation to the π-scheme sequence.

This was done for the two schemes reported below. For the π-scheme arbitrary rate constants were assigned. For the model on the left, without transitions between



Scheme 5a b

states with the same conductance, the reverse calculation (following the outlines of Appendix I) was made, to get the rate constants which reproduce the same global distributions. The quoted values were obtained in this way.

Afterwards, 12 000 consecutive events were generated for each model by feeding a computer with the parameters indicated in the schemes, and log-binned histograms (Sigworth and Sine 1987) of both open and closed dwell times were plotted in Fig. 4a and b respectively. Owing to our choice of rate constants, the global distributions overlap exactly. In contrast, the distributions of openings preceded by closings longer than 0.1 ms (Fig. 4c) clearly differ for the two models: they contain the same exponential components but with different weights. The same holds true for the distributions of closings preceded by openings longer than 1 ms (Fig. 4d). The conditional distributions for the two models contain the same exponential components but with different weights.

To conclude, the use of conditional distributions enables us to detect the hidden transitions. Therefore, even though exactly the opposite has been suggested (Bauer et al. 1987), when analysing experimental data is not correct to set to zero the rate constants of the paths which connect states in the same aggregate.

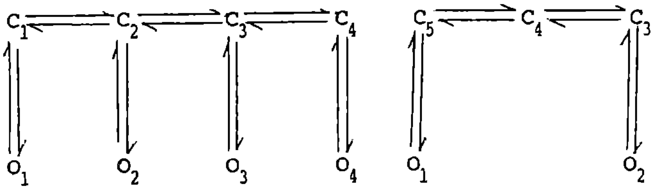
Concluding remarks

It is often assumed (Bauer et al. 1987; Crouzy and Sigworth 1990) that every coupled Markov scheme is statistically indistinguishable from a suitable uncoupled scheme. Kienker (1989), on the other hand, who showed how to use similarity transformations to obtain the uncoupled scheme equivalent to a coupled one, underlined that the validity of the obtained scheme has to be checked, by requiring that all the rate constants of the equivalent scheme are non-negative. Kienker stated that "it is not obvious exactly how this constrains S [the transformation matrix] in the general case".

For the particular case we have considered (the π-scheme) the equivalence with an uncoupled scheme only holds formally.

It makes sense to ask if the π-scheme is a very particular case and if it is possible to design a strategy to detect hidden transitions in more general cases.

Let us confine ourselves to schemes with hidden transitions in the closed aggregate but not in the open aggregate and assume that the transitions from open to open states occur through a chain of at least two closed states (i.e. we exclude simple pathways open-closed-open between different open states). Two typical schemes which satisfy these conditions are shown below:



Scheme 6a **b**

We can affirm, on a qualitative and general basis, that in this class of schemes the existence of hidden transitions can be detected by analysing the experimental data.

Consider Scheme (6a). The closings which occur after an opening in state O_2 and exit to state O_1 have a non-monotonic distribution. The system cannot exit to O_1 immediately after entering C_2 because there is an intermediate transition. So the distribution must vanish for vanishingly small closed times. On the other hand, as the close time duration increases, it must eventually tend exponentially to zero. Therefore, the distribution of closed dwell times beginning in C_2 and ending in O_1 admits a maximum, as do the distributions f_{31} and f_{42} computed for the π-scheme.

This qualitative argument shows that the best way to detect hidden transitions by analysing experimental or simulated data, is to use doubly conditional distributions by selecting, for instance, closed times on the basis of the values of the previous and the succeeding open times. In this way, and if the open states are well separated, one obtains clearly peaked distributions showing the existence of pathways between states in the closed aggregate.

Fredkin et al. (1985) showed that in the general scheme with n_o open states and n_c closed states the number of independent equations which can be obtained by using conditional distributions is equal to $2n_o n_c$, while the parameters which characterize the system are the $2n_o n_c$ rate constants for the transitions between open and closed states, plus the rate constants for transitions inside each aggregate, which are $n_o(n_o-1)+n_c(n_c-1)$. So only for uncoupled schemes can all rate constants be identified.

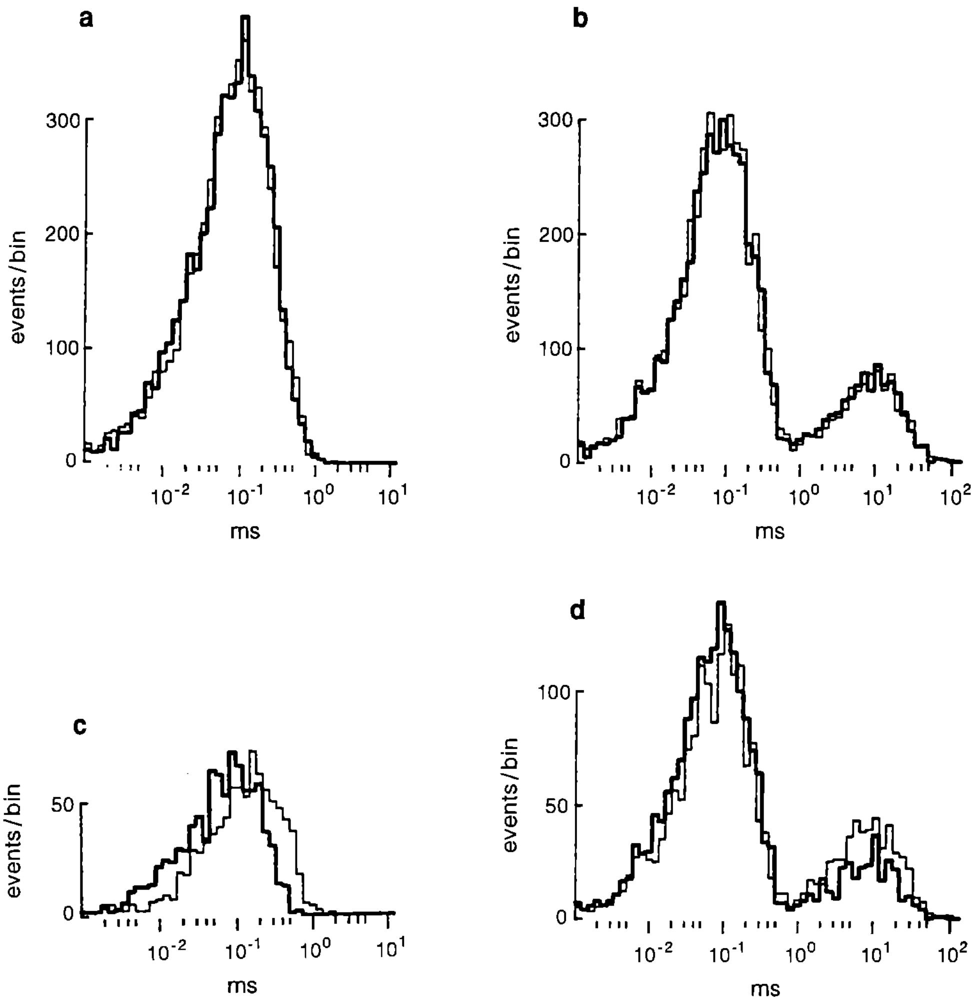


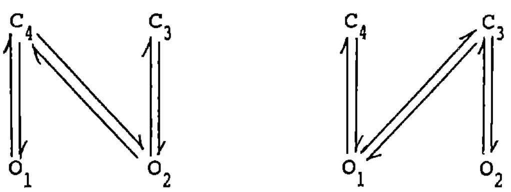
Fig. 4. Global (**a, b**) and conditional (**c, d**) distributions of dwell times generated by computer simulation of Schemes (5a) and (5b) given in the text. Note the log-binned scale for dwell times; in this representation an exponential distribution appears as a skewed bell-shaped curve (Sigworth and Sine 1983)

The possibility illustrated here to assess the existence of hidden transitions is not at odds with these results. It only shows that the solution of the system of independent equations becomes impossible if the space of the parameters is arbitrarily confined; for instance, by setting to zero the rate constants for transitions inside each aggregate.

The interpretation of ionic channel gating, as revealed by patch clamp recordings, in terms of Markov systems has recently been debated (Milhauser et al. 1988; Lauger 1988; Liebovitch and Sullivan 1987) and quite different models (for instance chaotic deterministic systems) have been suggested (Liebovitch 1989). However, the analysis of Markov systems and in particular the computation of conditional distributions is interesting also because it concerns these different frames of interpretation. In fact, the possibility of having a stringent test for Markov hypotheses (Barbi and Petracchi 1990) and using it on experimental data (Petracchi et al. 1991) is based on the use of conditional distributions. The most convincing test should be to be able to reconstruct the kinetic model from experimental data, as tried by an iterative simulation method by Magleby and Weiss (1990). The results presented herein give some contribution to this problem.

Appendix I

Let us consider the N-shaped uncoupled models shown below:



Scheme 7a **b**

Can such models account for any pair of global distributions with arbitrary time constants and weights? This appendix shows that the answer is yes and this result is used at the end of Sect. 2.

Let us firstly express, with reference to Scheme (7a), the time constants and weights of the global distributions in terms of the rate constants. The time constants are immediately obtained. Since the proability per unit time of leaving open state 1 (and so closing) is $k_{14}+k_{13}$, the open times from O_1 will be distributed exponentially with time constant $\Theta_1=(k_{14}+k_{13})^{-1}$. To get the two weights, consider the equilibrium probabilities P_i that the system is in state i ($i=1, \ldots, 4$). P_i equals the percentage dwell time in the corresponding state or, for a large number of identical systems, the percentage of systems in state i (at equilibrium). For each arm of a scheme without closed loops, in steady conditions, the mean frequency of forward transitions is identical to the mean frequency of reverse transitions (Colquhoun and Hawkes 1983; Hille 1984). This leads directly to the equations:

$$k_{13}\,P_1=k_{31}\,P_3 \qquad (A.1)$$
$$k_{32}\,P_3=k_{23}\,P_2$$

These yield the relation:

$$P_1/P_2=k_{31}\,k_{23}/k_{13}\,k_{32} \qquad (A.2)$$

On the other hand, since the time constants of openings coincide with the mean dwell times in the corresponding open states, we have:

$$\frac{P_1}{n_1\,\Theta_1}=\frac{P_2}{n_2\,\Theta_2}, \qquad (A.3)$$

where n_i is the number of dwell times in state i. By comparing the last two equations:

$$\frac{n_1}{n_2}=\frac{k_{31}\,k_{23}}{k_{13}\,k_{32}}\,\frac{\Theta_2}{\Theta_1} \qquad (A.4)$$

Now, going back to the original question, whereas the rate constants k_{23} and k_{41} of model (7a) are directly derived – as the reciprocals of Θ_2 and τ_4 respectively – from the exponential fit to the overall histograms, the other four rate constants must satisfy the following equations:

$$k_{31}+k_{32}=\tau_3^{-1} \qquad (A.5)$$
$$k_{13}+k_{14}=\Theta_1^{-1}$$
$$n_3/n_4=k_{13}(k_{32}+k_{31})/k_{14}\,k_{31}$$
$$n_1/n_2=k_{31}(k_{13}+k_{14})/k_{32}\,k_{13}$$

By introducing $u=k_{13}/k_{14}$ and $v=k_{32}/k_{31}$ the last two equations become:

$$n_3/n_4=u(1+v) \qquad (A.6)$$
$$n_1/n_2=(1+1/u)/v$$

which can easily be solved if the relation $n_1/n_2>n_4/n_3$ holds. If it is: $n_1/n_2<n_4/n_3$ the system (A.6) has no solutions, but in this case model (7b), which derives from (7a) by the exchange of states O_1 and O_2, C_3 and C_4 leads to a system like (A.6) with solutions. So the conclusion that N-models can account for any pair of global distributions is reached.

Appendix II

Herein the ratio $n_2/(n_1+n_2)=N_2$ for the π-shaped scheme (4a) will be computed. By calling P_i (as above) the probabilities of finding the system at any time in state i, detailed balance yields:

$$\frac{P_3}{P_4}=\frac{k_{43}}{k_{34}}\quad \frac{P_2}{P_3}=\frac{k_{32}}{k_{23}}\quad \frac{P_4}{P_1}=\frac{k_{14}}{k_{41}} \qquad (A.8)$$

whence:

$$\frac{P_2}{P_1}=\frac{k_{32}}{k_{23}}\,\frac{k_{43}}{k_{34}}\,\frac{k_{14}}{k_{41}} \qquad (A.9)$$

and being $n_2\,\Theta_2/n_1\,\Theta_1=P_2/P_1$ we get:

$$\frac{n_2}{n_1+n_2}=\frac{k_{32}\,k_{43}}{k_{34}\,k_{41}\,k_{32}\,k_{43}} \qquad (A.10)$$

Appendix III

a) Proof of the monotonicity of f_{32}

Let us firstly determine the position of $-(k_{41}+k_{43})$ with respect to the roots α and β of the characteristic equation (2.7). Hereafter we call α the greatest of the two roots, so that $|\alpha|<|\beta|$. Using simple algebra we can prove that $-(k_{41}+k_{43})$, substituted for s in the quadratic expression of (2.7), makes it negative. Moreover the coefficient of s^2 in that expression is positive, whence $-(k_{41}+k_{43})$ falls within the interval (β,α) and the coefficients of the exponentials in expression (2.10) of $f_{32}(t)$ are both positive.

b) Possible non monotonicity of f_3

First of all, we note that, by using for $\alpha\beta$ the expression coming from (2.7), function $f_3(t)$ can be written in the form:

$$f_3(t)=\frac{\alpha(\beta+k_{32})}{\alpha-\beta}\,e^{\alpha t}-\frac{\beta(\alpha+k_{32})}{\alpha-\beta}\,e^{\beta t} \tag{A.11}$$

Then we locate $-k_{32}$ with respect to the interval (β,α). Substitution of this value for s in the first member of (2.7) yields:

$$k_{32}^2-(k_{41}+k_{43}+k_{34}+k_{32})\,k_{32}+k_{41}\,k_{34}+k_{41}k_{32} + k_{43}\,k_{32}=k_{34}(k_{41}-k_{32}) \tag{A.12}$$

Therefore, for $k_{41}<k_{32}, -k_{32}$ falls inside the interval (β,α) and both coefficients in (A.11) are positive, so that $f_3(t)$ is monotonic.

In contrast, when $k_{41}>k_{32}$, $-k_{32}$ falls outside the interval (β,α). To be more precise, on the right of this interval, since the abscissa of the midpoint of the interval is, from (2.7):

$$(\alpha+\beta)/2=-(k_{41}+k_{43}+k_{34}+k_{32})/2<-k_{32} \tag{A.13}$$

Thus, in (A.11) the coefficient of the long lasting exponential, $\exp(\alpha t)$, is positive, whereas the coefficient of the short one, $\exp(\beta t)$, is negative, and smaller in absolute value. Monotonicity of $f_3(t)$ is assured when its starting slope is negative, i.e., by taking the time derivative for $t=0$, when:

$$\alpha^2(\beta+k_{32})-\beta^2(\alpha+k_{32})<0 \tag{A.14}$$

or:

$$\alpha\beta+k_{32}(\alpha+\beta)<0 \tag{A.15}$$

whence, by using (2.7) and simple algebra:

$$k_{34}(k_{41}-k_{32})<k_{32}^2 \tag{A.16}$$

When (A.16) is not satisfied, $f_3(t)$ presents a maximum.

It is easy to check that with the parameters of Fig. 1 $f_3(t)$ has a maximum, while with the parameters of Fig. 4 it is a monotonic function.

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