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Superposition Properties of Interacting Ion Channels

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ABSTRACT Quantitative analysis of patch clamp data is widely based on stochastic models of single-channel kinetics. Membrane patches often contain more than one active channel of a given type, and it is usually assumed that these behave independently in order to interpret the record and infer individual channel properties. However, recent studies suggest there are significant channel interactions in some systems. We examine a model of dependence in a system of two identical channels, each modeled by a continuous-time Markov chain in which specified transition rates are dependent on the conductance state of the other channel, changing instantaneously when the other channel opens or closes. Each channel then has, e.g., a closed time density that is conditional on the other channel being open or closed, these being identical under independence. We relate the two densities by a convolution function that embodies information about, and serves to quantify, dependence in the closed class. Distributions of observable (superposition) sojourn times are given in terms of these conditional densities. The behavior of two channel systems based on two- and three-state Markov models is examined by simulation. Optimized fitting of simulated data using reasonable parameters values and sample size indicates that both positive and negative cooperativity can be distinguished from independence.

INTRODUCTION

Ion channels are specialized proteins that control movement of ions across cell membranes. Direct observation of single-channel currents with the patch clamp technique has promoted the quantitative study of channel kinetics, which in turn has stimulated development of appropriate models. These models, which are usually based on stochastic process theory, enable proposed kinetic mechanisms to be studied by simulation; they also provide the basis for inferential analysis of experimental data.

Most ion channel modeling has been concerned with the behavior of a single channel acting in isolation. However, membrane patches from which recordings are made often contain more than one active channel; indeed, some channels appear to consist of a number of identical co-channels in the one macromolecular complex (Fox, 1987). In this setting, interpretation of the patch clamp record requires an understanding of how individual channel (or co-channel) processes combine to give the observed superposition process. This integration is simplified if channels are considered to act independently (Kijima and Kijima, 1987b; Yeo et al., 1989; Colquhoun and Hawkes, 1990; Fredkin and Rice, 1991), an assumption that was supported by early studies on certain channels (Hill and Chen, 1971a, b; Neher et al., 1978; Sigworth, 1980; Miller, 1982). However, there is more recent evidence that significant channel interaction may occur in some systems. For example, observed frequencies of simultaneous openings are highly suggestive of cooperativity (Yeramian et al., 1986; Hunter and Giebisch, 1987; Matsuda, 1988; Hymel et al., 1988; Schreibmayer et al., 1989), as are the gating kinetics of potassium channel subunits in *Xenopus* oocytes (Tytgat and Hess, 1992), the behavior of cardiac gap junctions in chick embryos (Chen and DeHaan, 1992), and the comparative properties of acetylcholine monomers and dimers (Schindler et al., 1984). Nonindependence may also explain deviations from the binomial distribution of steady-state probabilities of the number of open channels, as commented upon by several authors (Kiss and Nagy, 1985; Iwasa et al., 1986; Krouse et al., 1986; Queyroy and Verdetti, 1992).

In order to analyze such multichannel systems, a theoretical framework is required which has at basis some physically reasonable mechanism of cooperativity, yet remains mathematically and computationally tractable. It should provide a method of detecting and preferably quantifying in some way channel interaction, as well as characterizing it in terms of underlying single-channel distributional properties or kinetic parameters. Ideally, a comprehensive theory would enable estimation of these parameters from a superposition record.

In this paper we present kinetic models incorporating cooperative action between two identical channels. Each channel is modeled by a continuous-time Markov chain in which specified transition rates are made instantaneously dependent on whether the other channel is open or closed. The superposition of the two single-channel models, which we refer to as the complete process, is also a continuous-time Markov chain but on a larger state space. Conditional sojourn time densities are derived and related by convolution with functions that embody information about and quantify the dependence. Analytical solutions are presented and compared with the results from simulations. Other work concerned with

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modeling ion channel interactions can be found in Queyroy and Verdetti (1992), Lui and Dilger (1993), and Draber et al. (1993).

THEORY

Consider an isolated ion channel, where "isolated" is intended to guarantee that the behavior of the channel is not influenced in any way by the presence or behavior of neighboring channels. It is assumed that the channel has two experimentally distinguishable states, unit conducting (open) and nonconducting (closed); then, under equilibrium conditions it will exhibit some natural kinetic behavior observable as alternating open and closed periods. In the usual way, we assume that this kinetic behavior can be modeled by a continuous-time Markov chain in which the (finite) state space is aggregated into two classes, O (open) and C (closed) with n_O and n_C states, respectively. Then successive open times are identically distributed with distribution function, e.g., $F_X(t)$, mean μ_x, and density function $f_X(t)$; likewise, successive closed times are identically distributed with distribution function $F_Y(t)$, mean μ_Y, and density function $f_Y(t)$. (For any sojourn time T, we denote its distribution function by $F_T(t)$, density $f_T(t)$, and mean $\mu_T = \int_0^\infty \bar{F}_T(u)du$ where $\bar{F}_T(t) = 1 - F_T(t)$.) From standard Markov theory, assuming distinctness of eigenvalues, $f_X(t)$ and $f_Y(t)$ are each linear combinations of exponential densities, having nonnegative coefficients under detailed balance (Kijima and Kijima, 1987a).

We now allow a second, identical channel to exist in the neighborhood of the first, and consider the properties of the superposition signal (that is, a patch clamp recording) arising from concurrent activity in the two channels (see Fig. 1). If the two channels are independent, the distributions of the various types of observable sojourn times (Z_{1OO} and so on) in terms of the distributional properties of the single-channel sojourn times are given by Eqs. 24 and 25 of Yeo et al., (1989). The main focus of the remainder of this paper is the case in which the two channels are not independent in the following sense: the closed-time density (and similarly, the open-time density) of each channel is allowed to be dependent on whether the other channel is open or closed. Specified transition rates, and hence density parameters, are assumed to change instantaneously whenever the other channel opens or closes. The single-channel behavior is now characterized by conditional density functions $f_{Y|C}(t)$, $f_{Y|O}(t)$, $f_{X|C}(t)$, and $f_{X|O}(t)$, where, for example, $f_{Y|C}(t)$ is the density of closed sojourn times in either channel, given that the other channel is closed throughout. As a way of relating these conditional densities to each other we define convolution functions $k_C(t)$ and $k_O(t)$ $(0 \leq t < \infty)$ satisfying

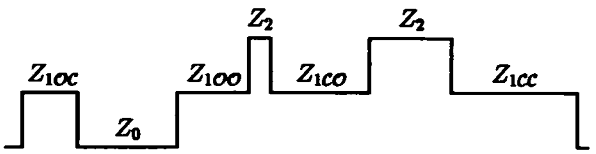

FIGURE 1 Idealized segment of a superposition signal from a patch containing two active channels (see also Yeo et al., 1989), showing notation for different types of sojourn time at conductance levels 0, 1, and 2; for example, Z_{1oc} denotes a sojourn time at level 1 (one channel open) that begins with an opening and ends with a closure.

$$f_{Y|O}(t) = k_C(t) * f_{Y|C}(t) \qquad f_{X|C}(t) = k_O(t) * f_{X|O}(t) \qquad (1)$$

where * denotes convolution, that is, $k(t) * f(t) = \int_0^{+\infty} k(t-u)f(u)\,du$. The conditional densities are properly normalized when $\int_{0-}^{+\infty} k(t)\,dt = 1$. The functions $k_C(t)$ and $k_O(t)$ describe how the closed-time density and open-time density, respectively, in one channel differs according to whether the other channel is open or closed throughout. If, say, the closed times are independent of the state of the other channel process, then $f_{Y|C}(t) \equiv f_{Y|O}(t)$ ($\equiv f_Y(t)$, but note that $f_{Y|C}(t) \equiv f_{Y|O}(t)$ does not necessarily imply the channels behave independently) and $k_C(t) = \delta(t)$, the Dirac δ function. Because of this last possibility it is preferable to work with the corresponding integrals $K_C(t) = \int_{0-}^{t} k_C(u)\,du$ and $K_O(t) = \int_{0-}^{t} k_O(u)\,du$.

As the Laplace transform of a convolution is a product of Laplace transforms and each conditional density of a sojourn time in the closed (or open) class is a linear combination of exponentials, it follows from Eq. 1 that

$$K_C(t) = 1 - \sum_{i=1}^{r_C} w_i e^{-m_i t} \qquad (0 \leq t < \infty) \qquad (2)$$

where $r_C \leq 2n_C - 1$, w_i can be less than, equal to, or greater than zero, and the exponents m_i are positive, with an analogous result for $K_O(t)$. Although $\int_{0-}^{\infty} k_C(u)\,du = 1$, $k_C(t)$ is not in general a density function (it may have jumps or take negative values); let $\bar{K}_C(t) = 1 - K_C(t)$ and define (by analogy with μ_T) $\mu_C = \int_0^\infty \bar{K}_C(u)\,du = \sum_{i=1}^{r_C} w_i/m_i$. Then $\mu_C = \mu_{Y|O} - \mu_{Y|C}$ and $\mu_O = \int_0^\infty \bar{K}_O(u)\,du = \mu_{X|C} - \mu_{X|O}$, where $\mu_{Y|C}$, $\mu_{Y|O}$, $\mu_{X|C}$, and $\mu_{X|O}$ are the means of the conditional densities above.

These ideas are now illustrated with a simple example. Consider an isolated channel with one open and one closed state, having open times and closed times each exponentially distributed with parameters α and β, respectively. A second, identical channel is now posited in the neighborhood of the first, the two interacting in such a way that either channel has opening rate β' during a period in which the other channel is open, and β otherwise. The conditional densities can be written as

$$\begin{aligned} f_{Y|C}(t) &= \beta e^{-\beta t} \\ f_{X|C}(t) &= f_{X|O}(t) = \alpha e^{-\alpha t} \qquad (3) \\ f_{Y|O}(t) &= \beta' e^{-\beta' t} \end{aligned}$$

Using Eq. 1 (and, most easily, Laplace transforms; thus, the Laplace transform of $k_O(t)$ is 1 and that of $k_C(t)$ is

$\beta'(s + \beta)/(\beta(s + \beta'))$, s being the Laplace variable) we obtain

$$k_C(t) = (\beta'/\beta)\delta(t) + (1 - \beta'/\beta)\beta' e^{-\beta' t}$$

$$k_O(t) = \delta(t) \quad (4)$$

$$K_C(t) = 1 - (1 - \beta'/\beta)e^{-\beta' t} \qquad K_O(t) = 1$$

with $\mu_C = (1 - \beta'/\beta)/\beta'$ and $\mu_O = 0$. If the interaction between channels is such that one channel being open predisposes its neighbor to open (positive cooperativity), $\beta' > \beta$ and $K_C(t) > 1$. Similarly, in the case of negative cooperativity, $\beta' < \beta$ and $K_C(t) < 1$; in either instance $K_C(0) = \beta'/\beta$. The form of $K_C(t)$, plotted as a function of time scaled in units of $1/\beta'$, is shown in Fig. 2 for various values of β'/β. Of course, if the channels are independent, $\beta' = \beta$ and $K_C(t) = 1$.

We can derive the unconditional density $f_Y^{(2)}(t)$ of a closed sojourn time in the presence of the second channel (irrespective of whether that second channel is open or closed) by using the complete (four-state) process, obtaining

$$f_Y^{(2)}(t) = C_1\lambda_1 e^{-\lambda_1 t} + C_2\lambda_2 e^{-\lambda_2 t} \quad (5)$$

where

$$\lambda_1, \lambda_2 = \tfrac{1}{2}\{\alpha + \beta' + 2\beta \mp R\}$$

$$C_1 = 1 - C_2 = 0.5 + \frac{\alpha - \beta' + 2\beta'(\alpha + \beta)/(\alpha + \beta')}{2R}$$

and $R = [(\alpha + \beta')^2 + 4\beta(\beta - \beta')]^{1/2}$. Again, under independence $\beta' = \beta$; and hence, $R = \alpha + \beta$, $\lambda_1 = \beta$, and $C_1 = 1$, the density collapsing to monoexponential with parameter β, as expected.

The monotonic exponential form of $K_C(t)$ found in the above example is not preserved in more complex single-channel models where there may be more than one state in the open or closed classes, and interaction is such that any transition rate may be dependent on the conductance state of the neighboring channel. For example, suppose that a channel has isolated kinetics described by

$$1 \underset{q_{21}}{\overset{q_{12}}{\rightleftarrows}} 2 \underset{\alpha}{\overset{\beta}{\rightleftarrows}} 3 \qquad \text{(Scheme 1)}$$

where 1 and 2 are closed states, and 3 is an open state. The open times remain exponentially distributed with parameter α, while the closed times are distributed as a linear combination of two exponentials, given by the right side of Eq. 5 with

$$\lambda_1, \lambda_2 = \tfrac{1}{2}\{q_{12} + d_2 \pm [(q_{12} + d_2)^2 - 4\beta q_{12}]^{1/2}\}$$

$$C_1 = \frac{\beta - \lambda_2}{\lambda_1 - \lambda_2} \qquad C_2 = 1 - C_1 \quad (6)$$

where $d_2 = q_{21} + \beta$.

Now assume that a second, identical channel exists in the neighborhood of the first, the two interacting in such a way that when one channel is open, q_{12} changes to some new value q'_{12} (note that the complete process does not satisfy detailed balance). Then $f_{Y|C}(t) = \sum_{i=1}^{2} C_i\lambda_i e^{-\lambda_i t}$, and $f_{Y|O}(t) = \sum_{i=1}^{2} C'_i\lambda'_i e^{-\lambda'_i t}$ where the respective parameters can be obtained using Eq. 6 with q_{12} replaced by q'_{12}. From $f_{Y|C}(t)$, $f_{Y|O}(t)$ and Eq. 1, $K_C(t)$ can be determined by deconvolution and integration:

$$K_C(t) = 1 - \left(1 - \frac{q'_{12}}{q_{12}}\right) \times \left[\left(\frac{C'_1}{b_1}\right)e^{-\lambda'_1 t} + \left(\frac{C'_2}{b_2}\right)e^{-\lambda'_2 t} - \left(\frac{1}{b_3}\right)e^{-q_{12} t}\right] \quad (7)$$

where $b_i = 1 + (q_{12} - \beta)(q'_{12} - m_i)/(q_{12}q_{21})$, $i = 1, 2, 3,$

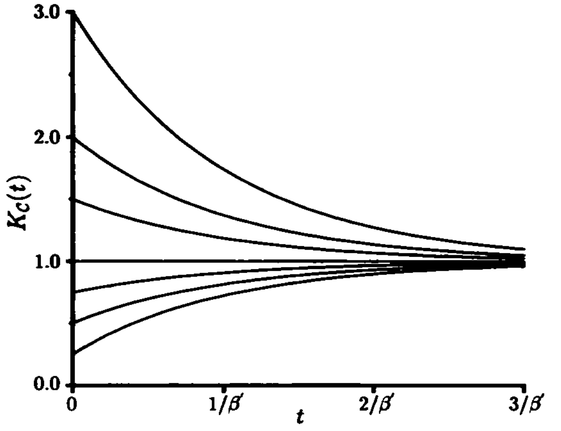

FIGURE 2 Interaction in a two-channel system where each channel is modeled as a two-state Markov process with opening rate β (respectively β') when the neighboring channel is closed (open). The function $K_C(t)$ relates single-channel closed time densities $f_{Y|C}(t), f_{Y|O}(t)$ conditional on the other channel being closed or open, respectively (see Eq. 3), and is plotted (from Eq. 4) for various values of β'/β (indicated by intercept $K_C(0)$) on a normalized time scale. Independence implies $\beta' = \beta$ and $K_C(t) = 1$.

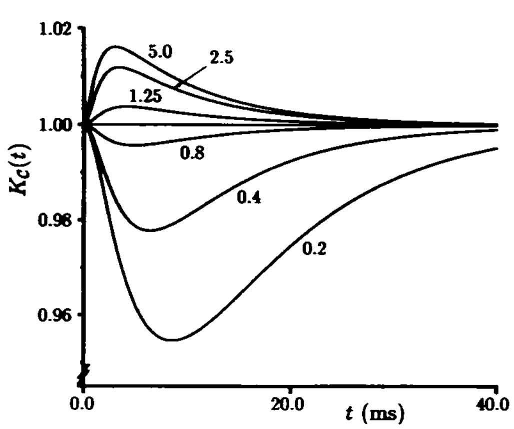

FIGURE 3 Interaction in a two-channel system where each channel is modeled as a three-state Markov process based on Scheme 1, and the transition rate q_{12} is dependent on the neighboring channel being closed (when it is q_{12}) or open (q'_{12}). Plots were generated using Eq. 7 with parameter values $q_{12} = 0.82$, $q_{21} = 0.023$, $\beta = 0.115$, $\alpha = 0.06\ \text{ms}^{-1}$, and a range of values for q'_{12} (indicated by the ratio q'_{12}/q_{12} written beside each curve).

and $m_1 = \lambda'_1$, $m_2 = \lambda'_2$, and $m_3 = q_{12}$. It can be shown that $C'_1/b_1 + C'_2/b_2 = 1/b_3$; thus, in this instance $K_C(0) = 1$. Also $\mu_C = (1 - q'_{12}/q_{12})q_{21}/(q'_{12}\beta)$. Clearly, under independence $q'_{12} = q_{12}$ and $K_C(t) = 1$. These results are illustrated in Fig. 3 where $K_C(t)$ for this model is plotted for various values of q'_{12}/q_{12}. Regardless of independence, $K_O(t) = 1$.

Suppose now that it is the opening rate β that changes (e.g., to β') when the other channel is open (in which case the complete system satisfies detailed balance). Then $K_O(t) = 1$ and, using methods similar to those giving Eq. 7,

$$K_C(t) = 1 - (1 - \beta'/\beta)[C'_1 e^{-\lambda'_1 t} + C'_2 e^{-\lambda'_2 t}] \qquad (8)$$

where λ'_1, λ'_2, C'_1, and C'_2 are obtained from Eq. 6 with β replaced by β' and d_2 replaced by $d'_2 = q_{21} + \beta'$. Note that $K_C(0) = \beta'/\beta$ and the homology with Eq. 4; also $\mu_C = (1 - \beta'/\beta)(q_{12} + q_{21})/(q_{12}\beta')$.

We now consider what is experimentally observable in such a two-channel system, that is, sojourn times of the types illustrated in Fig. 1. The distributions of these sojourn times can be obtained using properties of the transition rate matrix of the complete process and conditional independence, allowing us to generalize Eqs. 24 and 25 of Yeo et al. (1989) as

$$\bar{F}_{Z_0}(t) = \bar{F}_{Y|C}(t)\bar{H}_{Y|C}(t) \qquad \bar{F}_{Z_2}(t) = \bar{F}_{X|O}(t)\bar{H}_{X|O}(t) \qquad (9)$$

$$\begin{aligned} f_{Z_{1OO}}(t) &= \nu_{OO}^{-1}\,\bar{F}_{X|C}(t)\{-d[\bar{H}_{Y|O}(t)]/dt\} \\ f_{Z_{1CC}}(t) &= \nu_{CC}^{-1}\,\bar{F}_{Y|O}(t)\{-d[\bar{H}_{X|C}(t)]/dt\} \\ f_{Z_{1OC}}(t) &= \nu_{OC}^{-1}\,f_{X|C}(t)\bar{H}_{Y|O}(t) \\ f_{Z_{1CO}}(t) &= \nu_{CO}^{-1}\,f_{Y|O}(t)\bar{H}_{X|C}(t) \end{aligned} \qquad (10)$$

where the normalizing constants $\nu_{..}$ are such that the functions integrate to unity, and, for example, $\bar{H}_{Y|O}(t)$ is the probability that a channel remains closed for at least time t, given that the other channel has just opened and remains open for at least time t. In the case of independent channels, this is the tail distribution function of a closed sojourn from a random time point ("a remaining sojourn time"), which would satisfy $\bar{H}_{Y|O}(t) = \mu_{Y|O}^{-1} \int_t^\infty \bar{F}_{Y|O}(u)\,du\ (\equiv \bar{H}_{Y|C}(t))$, consistent with standard renewal theory; furthermore, $f_{Z_{1OO}}(t) = f_{Z_{1CC}}(t)$, $t \geq 0$.

These results for independent channels apply virtually unchanged in certain dependent channel models, and we restrict consideration here and in the next section to such special cases (the more general theory with applications will be dealt with elsewhere). An example is the two-state channel system above, in which any closed (or open) sojourn, or remaining sojourn, has a single exponential distribution. Another example is the three-state model of Scheme 1, provided that only the channel opening rate can change when the other channel changes conductance level (the situation is different if q_{12} can change). For these special cases,

$$\nu_{OO} = \eta/\mu_{Y|O} \qquad \nu_{CC} = \eta/\mu_{Y|C}$$

$$\nu_{OC} = (\mu_{Y|O} - \eta)/\mu_{Y|O} \qquad \nu_{CO} = (\mu_{X|C} - \eta)/\mu_{X|C}$$

where $\eta = \int_0^\infty \bar{F}_{Y|O}(u)\bar{F}_{X|C}(u)\,du$; hence, $f_{Z_{1OO}}(t) = f_{Z_{1CC}}(t) = \bar{F}_{X|C}(t)\bar{F}_{Y|O}(t)/\eta$.

Steady-state probabilities p_0, p_1 and p_2 that the two-channel system is in each of the three conductance levels 0, 1, and 2, respectively, can be derived using the transition rate matrix of the complete process. For Scheme 1 this can be reduced to six states (Colquhoun and Hawkes, 1990) corresponding to one channel being in state i and the other in state j ($i, j = 1, 2, 3$), namely (1, 1), (1, 2), (2, 2), (1, 3), (2, 3), and (3, 3), the first three for level 0, the next two for level 1, and the last for level 2. A full discussion will be given elsewhere. This six-state model may be approximated, in the case where β changes to β' when the other channel opens, by a three-state continuous-time Markov chain on the conductance levels 0, 1, and 2, with

$$0 \underset{\alpha}{\overset{2\bar{\beta}}{\rightleftarrows}} 1 \underset{2\alpha}{\overset{\bar{\beta}'}{\rightleftarrows}} 2 \qquad \text{(Scheme 2)}$$

Choosing $\bar{\beta}$ and $\bar{\beta}'$ so that this approximate process has the same steady-state probabilities as the complete process we find $\bar{\beta} = \beta q_{12}/(q_{12} + q_{21})$, $\bar{\beta}' = \beta' q_{12}/(q_{12} + q_{21})$ and $p_0 = \alpha^2/h$, $p_1 = 2\alpha\bar{\beta}/h$ and $p_2 = \bar{\beta}\bar{\beta}'/h$, where $h = \alpha^2 + 2\alpha\bar{\beta} + \bar{\beta}\bar{\beta}'$. Note that $\bar{\beta} = 1/\mu_{Y|C}$, $\bar{\beta}' = 1/\mu_{Y|O}$ and $\alpha = 1/\mu_{X|C} = 1/\mu_{X|O}$.

SIMULATIONS

Two-channel superposition records were simulated for a selection of single-channel Markov models, with and without interaction of the form described above. The distributions pertaining to the six types of sojourn time (Fig. 1) were estimated from binned data. Steady-state probabilities were computed as the ratio of the total duration of a particular sojourn type to the duration of the whole record. From all these estimates, using Eqs. 9 and 10 and the predicted sojourn type probabilities, the decay rates and weights of the exponentials of the four conditional density functions of Eq. 1 were estimated by nonlinear least-squares regression (Nelder and Mead, 1965). The functions $K_C(t)$ and $K_O(t)$ were then obtained from Eq. 1 by deconvolution and integration.

For the two-state single-channel model (see Fig. 2 for analytical results) with $\alpha = 1.0$ and $\beta = 2.0\ \mathrm{ms}^{-1}$, the simulated steady-state probabilities of zero, one, or two channels being open are 0.1105, 0.4442, and 0.4453 if the channels are independent ($\beta' = \beta$, see Eq. 3). As expected, these probabilities shift in the presence of cooperativity; for example, when $\beta' = 3\ \mathrm{ms}^{-1}$ they are 0.0911, 0.3635, and 0.5454 (very close to the theoretical values of 1/11, 4/11, and 6/11, respectively). A segment of the simulated superposition process for the above two cases is illustrated in Fig. 4. Estimates of $K_C(t)$ and $K_O(t)$ obtained from the simulated record are plotted in Fig. 5 (dashed lines) for the cases $\beta' = 3.0$, 2.0, and 1.75 ms^{-1}. In all cases they are essentially indistinguishable from the functions computed analytically using Eq. 4 from knowledge of the underlying single-channel parameters.

We return in Fig. 6 to the two-channel system where an isolated single-channel model is based on Scheme 1 with

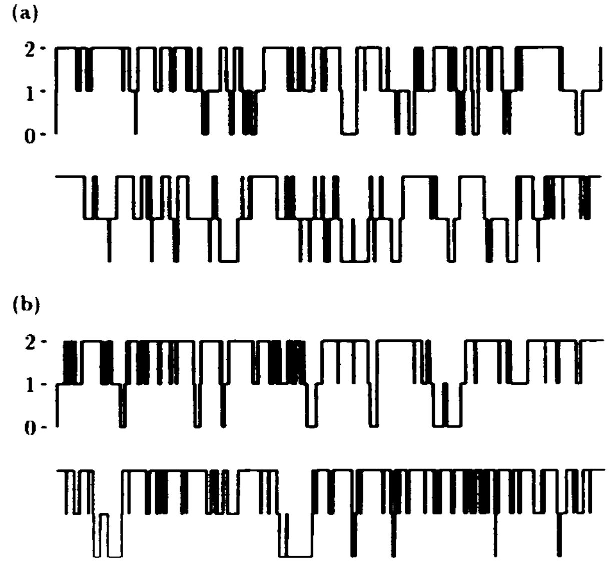

FIGURE 4 A continuous segment (64 ms) of the simulated superposition process for the two-channel, two-state system of Fig. 2 with $\alpha = 1.0$, $\beta = 2.0\ \mathrm{ms}^{-1}$. (*a*) independent channels (i.e., $\beta' = 2.0\ \mathrm{ms}^{-1}$); (*b*) positively cooperative channels ($\beta' = 3.0\ \mathrm{ms}^{-1}$).

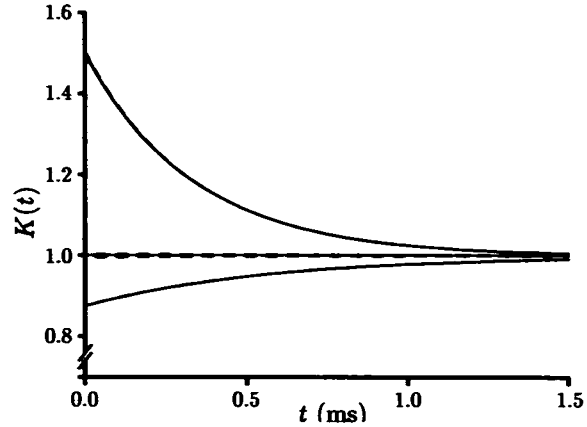

FIGURE 5 Functions $K_C(t)$ and $K_O(t)$ (denoted collectively $K(t)$ here and in following figures) estimated from simulated data (consisting of 50,000 sojourns for each of the six types, *dashed lines*) and derived analytically (from Eq. 4, *solid lines*) for the two-channel, two-state system of Fig. 2 with $\alpha = 1.0$, $\beta = 2.0\ \mathrm{ms}^{-1}$. Curves from above downwards represent $K_C(t)$ for $\beta' = 3.0\ \mathrm{ms}^{-1}$ (positive cooperativity), $\beta' = 2.0\ \mathrm{ms}^{-1}$ (independence) and $\beta' = 1.75\ \mathrm{ms}^{-1}$ (negative cooperativity), respectively. $K_O(t) = 1$ for all cases.

parameter values given in Fig. 3, and with interaction via the opening rate from β ($= 0.115\ \mathrm{ms}^{-1}$) to some new value β' while the neighboring channel is open. When $\beta' = 0.375\ \mathrm{ms}^{-1}$ (upper traces Fig. 6) the estimate for $K_C(t)$ (dashed curve) is very close to the analytical solution (solid curve). With negative cooperativity ($\beta' = 0.0575\ \mathrm{ms}^{-1}$, lower traces) $K_C(t)$ is slightly underestimated at low values of t, reflecting difficulty in fitting fast exponentials and a relatively small sample size (collected by considering sufficient of a simulated record segment to give at least 100 sojourns in each of the six types). A similar difficulty is evident in

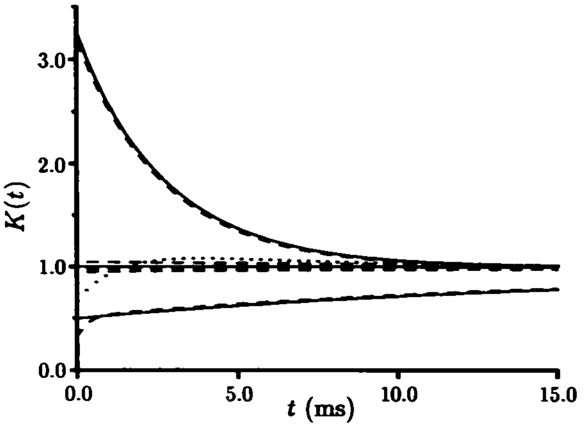

FIGURE 6 Interaction in a two-channel system where each channel is modeled according to Scheme 1, and the single channel opening rate is dependent on the neighboring channel being closed (when it is β) or open (β'). Using parameter values given in Fig. 3, $K_C(t)$ and $K_O(t)$ were estimated from simulated data consisting of a minimum of 100 sojourns per type (*dashed and dotted lines*) and by solution of Eq. 8 (*solid lines*) for the cases $\beta' = 0.375\ \mathrm{ms}^{-1}$ [*upper traces*: $K_C(t)$], $\beta' = 0.0575\ \mathrm{ms}^{-1}$ [*lower traces*: $K_C(t)$] and $\beta' = 0.115\ \mathrm{ms}^{-1}$ [*dotted trace*: $K_C(t)$]. $K_O(t)$ is grouped around $K(t) = 1$ in all cases.

$K_C(t)$ under independence (dotted curve), but in all cases $K_O(t)$ is well estimated (dashed lines close to $K(t) = 1$).

Fig. 7 presents simulation results and analytical solutions for $K_C(t)$ and $K_O(t)$ in a special case of the two-state model (see Fig. 5) where the symmetry of the rate constants under closed ($\alpha = 1.0$, $\beta = 2.0\ \mathrm{ms}^{-1}$) and open ($\alpha' = 2.0$, $\beta' = 4.0\ \mathrm{ms}^{-1}$) conditions renders superposition data insensitive to tests for nonindependence based on steady-state probabilities and binomial theory. However, estimation of $K_C(t)$ and $K_O(t)$ from simulated data (again with a minimum of 100 sojourns) resulted in well defined functions very close to the analytical solutions.

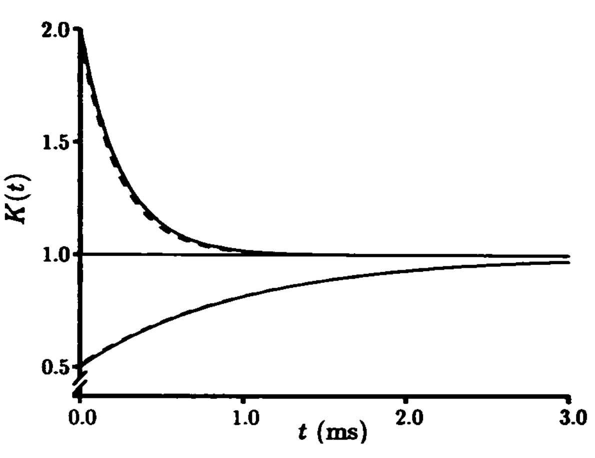

FIGURE 7 Interaction in a two-channel, two-state system where both opening and closing rates are dependent on the neighboring channel being closed (when they are β and α, respectively) or open (β' and α', see text). Estimates from simulated data (minimum of 100 sojourns, *dashed lines*) and analytical solutions (*solid lines*) are given for $K_C(t)$ (*upper traces*) and $K_O(t)$ (*lower traces*).

DISCUSSION

The possibility of interaction between ion channels is important for several reasons. Independence or nonindependence in this sense is relevant in any integrative approach to understanding macroscopic membrane phenomena based on single-channel behavior. Conversely, this is equally important in statistical analysis of macroscopic signals for elucidation of underlying single molecular events; examples of this include interpretation of concurrent openings in patch clamp records, and membrane noise analysis. In any event, the question of cooperativity between channels has considerable intrinsic interest in relation to macromolecular properties and transmembrane signaling in general. Moreover, if cooperativity does exist, it might potentially be exploited pharmacologically.

There appears to be accumulating evidence that interaction does occur in some systems. This is usually inferred from some discrepancy between observed behavior and the predictions of an independence assumption. Although this is an appropriate initial step in analysis, and some of these tests are quite sophisticated (Dabrowski et al., 1990), they are not necessarily conclusive or quantitative (Uteshev, 1993), nor based upon possible mechanisms of cooperativity, and therefore cannot serve in model testing. The approach in this paper has been to propose a simple mechanism for interaction and to examine consequential properties of the observable process.

The mechanism studied here postulates that the isomerization involved in changing between conducting and nonconducting states in one channel can result in an essentially instantaneous change in particular rate constants in a neighboring channel. If the two channels exist within a single macromolecular complex (that is, they are co-channels), this could occur through allosteric coupling. In the case of separate but contiguous channels the effect could be mediated by electrical field alterations associated with conformational change. The assumption of instantaneity is not unreasonable, given the known time scale of gating isomerization in these molecules. It should be noted that the model need not be restricted to conditioning on one channel being open or closed; in theory, occupation of any state could alter behavior in the other channel, although relating model results to experimental data may be more difficult.

In the Markov framework used to model the single-channel process, it is natural to express interaction in terms of an alteration in specified transition rates, which themselves relate to identifiable chemical kinetic processes such as agonist binding or channel gating. If, as in examples presented here, these are transition rates within or out of the closed class, their alteration will affect the distribution of closed times. Thus, the distributions of closed times conditional on an event of interest (here, the neighboring channel being open or closed) can be compared, with their difference being some indication and measure of the interaction. It is convenient to make this comparison using a convolution relation which provides a function $K_C(t)$ that quantifies this difference; in the two- and three-state models examined where only the opening rate constant may change, $K_C(t)$ is less than, equal to, or greater than unity in the case of negative, zero (independence), and positive cooperativity, respectively. (The function $K_O(t)$ is similarly derived and has analogous behavior.) Using simulated data, $K_C(t)$ and $K_O(t)$ have been estimated from superposition records by optimized fitting. For the models examined, these estimates have been almost exactly those predicted analytically in the case of large sample size (more than 50,000 sojourns/type), with only small deviations when using samples consisting of a minimum of 100 sojourns/type.

Although we have only considered some simple two-channel models of interaction, various extensions would not seem insurmountable in principle. Allosteric coupling across several co-channels within the same macromolecular complex is quite feasible. In the case of multiple spatially separated (but electrostatically interacting) channels, the possibility of time dependence or of distance-dependent delay or attenuation of cooperative responses needs to be considered. A further extension concerns L-type calcium channels, where it appears to be the localized accumulation of calcium ions following activation (rather than the conductance change per se) that mediates interchannel dependence (DeFelice, 1993).

We are currently developing a general theory for aggregated, interacting Markov chains applicable to more complex models having larger state space and channel number than in the examples presented here.

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REFERENCES

Chen, Y., and R. L. DeHaan. 1992. Multiple-channel conductance states and voltage regulation of embryonic chick cardiac gap junctions. *J. Membr. Biol.* 127:95–111.

Colquhoun, D., and A. G. Hawkes. 1990. Stochastic properties of ion channels and bursts in a membrane patch that contains two channels: evidence concerning the number of channels present when a record containing only single openings is observed. *Proc. R. Soc. Lond. B. Biol. Sci.* 240: 453–477.

Dabrowski, A. R., D. McDonald, and U. Rösler. 1990. Renewal theory properties of ion channels. *Ann. Stat.* 18:1091–1115.

DeFelice, L. J. 1993. Molecular and biophysical view of the Ca channel: a hypothesis regarding oligomeric structure, channel clustering, and macroscopic current. *J. Membr. Biol.* 133:191–202.

Draber, S., R. Schultze, and U. Hansen. 1993. Cooperative behaviour of K^+ channels in the tonoplast of *Chara corallina*. *Biophys. J.* 65:1553–1559.

Fox, J. A. 1987. Ion channel subconductance states. *J. Membr. Biol.* 97:1–8.

Fredkin, D. R., and J. A. Rice. 1991. On the superposition of currents from ion channels. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 334:347–356.

Hill, T. L., and Y. D. Chen. 1971a. On the theory of ion transport across the nerve membrane. II. Potassium ion kinetics and cooperativity (with $x = 4$). *Proc. Natl. Acad. Sci. USA.* 68:1711–1715.

Hill, T. L., and Y. D. Chen. 1971b. On the theory of ion transport across the nerve membrane. III. Potassium ion kinetics and cooperativity (with $x = 4, 6, 9$). *Proc. Natl. Acad. Sci. USA.* 68:2488–2492.

Hunter, M., and G. Giebisch. 1987. Multi-barrelled K channels in renal tubules. *Nature.* 327:522–524.

Hymel, L., J. Striessnig, H. Glossmann, and H. Schindler. 1988. Purified skeletal muscle 1,4-dihydropyridine receptor forms phosphorylation-dependent oligomeric calcium channels in planar bilayers. *Proc. Natl. Acad. Sci. USA.* 85:4290–4294.

Iwasa, K., G. Ehrenstein, N. Moran, and M. Jia. 1986. Evidence for interactions between batrachotoxin-modified channels in hybrid neuroblastoma cells. *Biophys. J.* 50:531–537.

Kijima, S., and H. Kijima. 1987a. Statistical analysis of channel current from a membrane patch. I. Some stochastic properties of ion channels or molecular systems in equilibrium. *J. Theor. Biol.* 128: 423–434.

Kijima, S., and H. Kijima. 1987b. Statistical analysis of channel current from a membrane patch. II. A stochastic theory of a multi-channel system in the steady-state. *J. Theor. Biol.* 128:435–455.

Kiss, T., and K. Nagy. 1985. Interaction between sodium channels in mouse neuroblastoma cells. *Eur. Biophys. J.* 12:13–18.

Krouse, M. E., G. T. Schneider, and P. W. Gage. 1986. A large anion-selective channel has seven conductance levels. *Nature.* 319:58–60.

Lui, Y., and J. P. Dilger. 1993. Application of the one- and two- dimensional Ising models to studies of cooperativity between ion channels. *Biophys. J.* 64:26–35.

Matsuda, H. 1988. Open-state substructure of inwardly rectifying potassium channels revealed by magnesium block in guinea-pig heart cells. *J. Physiol. Lond.* 397:237–258.

Miller, C. 1982. Open-state substructure of single chloride channels from *Torpedo electroplax. Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 299: 401–411.

Neher, E., B. Sakmann and J. H. Steinbach. 1978. The extracellular patch clamp: a method for resolving currents through individual open channels in biological membranes. *Pflügers Arch.* 375:219–228.

Nelder, J. A., and R. Mead. 1965. A simplex method for function minimization. *Computer J.* 7:308–313.

Queyroy, A., and J. Verdetti. 1992. Cooperative gating of chloride channel subunits in endothelial cells. *Biochim. Biophys. Acta.* 1108:159–168.

Schindler, H., F. Spillecke and E. Neumann. 1984. Different channel properties of *Torpedo* acetylcholine receptor monomers and dimers reconstituted in planar membranes. *Proc. Natl. Acad. Sci. USA.* 81:6222–6226.

Schreibmayer, W., H. A. Tritthart and H. Schindler. 1989. The cardiac sodium channel shows a regular substate pattern indicating synchronized activity of several ion pathways instead of one. *Biochim. Biophys. Acta.* 986:172–186.

Sigworth, F. J. 1980. The conductance of sodium channels under conditions of reduced current at the node of Ranvier. *J. Physiol. Lond.* 307:131–142.

Tytgat, J., and P. Hess. 1992. Evidence for cooperative interactions in potassium channel gating. *Nature.* 359:420–423.

Uteshev, V. 1993. The binomial distribution and the evidence for independent action of ion channels. *J. Theor. Biol.* 163:485–489.

Yeo, G. F., R. O. Edeson, R. K. Milne and B. W. Madsen. 1989. Superposition properties of independent ion channels. *Proc. R. Soc. Lond. B. Biol. Sci.* 238:155–170.

Yeramian, E., A. Trautmann and P. Claverie. 1986. Acetylcholine receptors are not functionally independent. *Biophys. J.* 50:253–263.