THE USE OF DWELL TIME CROSS-CORRELATION FUNCTIONS TO STUDY SINGLE-ION CHANNEL GATING KINETICS

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ABSTRACT The derivation of cross-correlation functions from single-channel dwell (open and closed) times is described. Simulation of single-channel data for simple gating models, alongside theoretical treatment, is used to demonstrate the relationship of cross-correlation functions to underlying gating mechanisms. It is shown that time irreversibility of gating kinetics may be revealed in cross-correlation functions. Application of cross-correlation function analysis to data derived from the locust muscle glutamate receptor-channel provides evidence for multiple gateway states and time reversibility of gating. A model for the gating of this channel is used to show the effect of omission of brief channel events on cross-correlation functions.

INTRODUCTION

The overall aim of kinetic analysis of single ion channel data (Horn, 1984) is to provide a complete description of the underlying gating mechanism of the ion channel in question, including estimates of all rate constants (transition probabilities). As demonstrated by Horn and Vandenberg (1984), maxium likelihood methods may be employed to discriminate between alternative gating mechanisms for a channel, and to provide parameter estimates. However, before applying such procedures, it is important to derive as much information as possible concerning likely gating mechanisms via statistical analysis of channel dwell (i.e., open and closed) times.

Several authors (Fredkin et al., 1985; Colquhoun and Hawkes, 1987; Ball and Sansom, 1988*a*,*b*) have discussed the use of single-channel dwell time autocorrelation functions to characterize branched and cyclic gating mechanisms. Such analysis has been applied to the *Torpedo* nicotinic acetylcholine receptor (Labarca et al., 1985) and to the locust muscle glutamate receptor (GluR) (Kerry et al., 1987, 1988), and has proved extremely valuable.

Here, we extend these studies to the use of dwell time cross-correlation functions to further characterize branched and cyclic mechanisms. Such cross-correlation functions therefore provide an alternative to the evaluation of frequencies of paired open-closed durations (Steinberg, 1987*b*). We illustrate this approach for two simple gating mechanisms. The effect of time irreversiblity on cross-correlation functions is analysed. We then present the results of applying such anaysis to data derived from the GluR, and discuss this in the light of a model which has proved useful in understanding the gating kinetics of the GluR. Finally, we consider the effects of time interval omission, arising from the finite response time of the single-channel recording and detection system, upon cross-correlation functions.

METHODS

Determination of Cross-Correlation Functions

Let the series of single channel-open and closed (dwell) times be represented by

$$t_o(1), t_c(1), \ldots, t_o(i), t_c(i), \ldots, t_o(m), t_c(m),$$

where $t_o(i)$ is the *ith* open time and $t_c(i)$ is the *ith* closed time, and m is the total number of single-channel events.

Note that we define an event as an opening plus the following closing of the channel. Here we will assume that the measurements are obtained under constant conditions and that the channel dwell time data have been obtained after the channel has been exposed to such conditions for an extended period of time, i.e., we are not concerned here with relaxation of ion channels after a step change in membrane potential or in agonist concentration (Ball et al., 1988).

The single-channel dwell time cross-correlation function is defined by

$$r_{oc}(k) = \mathrm{Cov}\,[t_o(i), t_c(i+k)]/(\mathrm{Var}\,[t_o(i)]\,\mathrm{Var}\,[t_c(i)\,])^{0.5}, \quad (1)$$

where Cov denotes covariance, Var denotes variance and the lag, k, may adopt both negative (closing preceeding the opening) and positive (closing succeeding the opening) integer values (Chatfield, 1980). The covariance is given by:

$$\mathrm{Cov}\,[t_o(i), t_c(i+k)] = E[(t_o(i) - E[t_o(i)])(t_c(i+k) - E[t_c(i)])], \quad (2)$$

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where $E[x]$ denotes the expectation of x. In the same nomenclature the open time variance, for example, is given by:

$$\mathrm{Var}\,[t_o(i)] = E[(t_o(i) - E[t_o(i)])^2]. \tag{3}$$

Both of these expressions may be simplified, in the usual manner, to give:

$$\mathrm{Cov}\,[t_o(i), t_c(i + k)] = E[t_o(i).t_c(i + k)] - E[t_o(i)].E[t_c(i)] \tag{4}$$

and (more familiarly):

$$\mathrm{Var}\,[t_o(i)] = E[t_o(i)^2] - (E[t_o(i)])^2. \tag{5}$$

The cross-correlation measures the degree of correlation between channel openings, $t_o(i)$, and channel closings, $t_c(i + k)$, separed by lag k. The cross-correlation function may therefore adopt either positive or negative values, dependent upon the nature of the correlation. Note that one may also define a cross-correlation between closings, $t_c(i)$, and openings, $t_o(i + k)$:

$$r_{co}(k) = \mathrm{Cov}\,[t_c(i), t_o(i + k)]/(\mathrm{Var}\,[t_c(i)]\ \mathrm{Var}\,[t_o(i)])^{0.5}. \tag{6}$$

For a stationary process, one can show that:

$$r_{co}(k) = r_{oc}(-k). \tag{7}$$

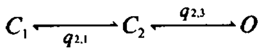
So, we may restrict ourselves to values of $k \geq 0$ by consideration of both $r_{oc}(k)$ and $r_{co}(k)$. A stationary process may or may not be time reversible. For time reversible processes (i.e., channel gating mechanisms at thermodynamic equilibrium), one can show that

$$r_{oc}(k) = r_{co}(k) \tag{8}$$

and hence only $r_{oc}(k)$, $k \geq 0$ need be considered. Conversely, if Eq. 8 does not hold, then one may conclude that the dwell time series is irreversible, and hence the underlying gating mechanism is not at thermo-dynamic equilibrium. Therefore, experimentally, it is worthwhile to evaluate both $r_{oc}(k)$ and $r_{co}(k)$, $k \geq 0$ in order to test for this latter possibility.

Simulation

Ion channel data was simulated using the algorithm described by Clay and DeFelice (1983), modified for time interval omission. The basis of this algorithm may be understood by consideration of the following mechanism



Let the channel be in state C_2. A pseudo-random number, r, from the unit rectangular variate is taken. The time spent in state C_2 before leaving, t, is given by

$$t = -\lambda \ln r, \tag{9}$$

where

$$\lambda = q_{2,1} + q_{2,3} \tag{10}$$

i.e., the sum of the rates for transitions by which the channel leaves C_2. The next state of the channel is chosen by selecting a further random number, s, also from the unit rectangular variate. If

$$s < q_{2,1}/(q_{2,1} + q_{2,3}) \tag{11}$$

then the next state of the channel is C_1. Otherwise, the next state of the channel is O. This algorithm is used to produce a set of open and closed times by noting the times at which open to closed and closed to open transitions occur. Time interval omission is imposed by post-processing of the resultant dwell time vector such that all channel sojourns $<\tau_o$ are discarded. This, and all other computations, were carried out using programs written in Fortran77, drawing on the NAG subroutine library for numerical algorithms.

Experimental Data.

The single-channel data from the locust muscle GluR was that described in previous publications from our laboratory (Kerry et al., 1987, 1988), where details of recording conditions and data reduction procedures may be found.

RESULTS

Theory

Channel dwell time cross-correlation functions can be derived from proposed gating mechanisms using the general framework described by Ball and Sansom (1988*a*, *b*). Following an outline of that framework, we develop here formulae for channel dwell time cross-correlation functions when time interval omission is present. Equivalent formulae for when time interval omission is ignored then follow as a special case.

Our starting point is to model a single-channel gating mechanism as a finite state–space, continuous time Markov chain $\{X(t); t \geq 0\}$ that is irreducible and time reversible. The states are labeled $1, 2, \ldots, n$, where states 1 to n_o are the open channel states, and states $n_o + 1$ to $n_o + n_c = n$ are the closed states.

For $i \neq j$ let q_{ij} be the transition rate of $\{X(t); t \geq 0\}$ from state i to state j. Let Q be the $n \times n$ matrix with off-diagonal elements q_{ij} and diagonal elements $q_{ii} = -\Sigma_{j \neq i} q_{ij}$. Partition the matrix Q into

$$Q = \begin{bmatrix} Q_{oo} & Q_{oc} \\ Q_{co} & Q_{cc} \end{bmatrix},$$

where, for example, Q_{oo} corresponds to the transition matrix within the open states, and define $n \times n$ matrices Q_0 and Q_1 by

$$Q_0 = \begin{bmatrix} Q_{oo} & 0 \\ 0 & Q_{cc} \end{bmatrix}$$

and

$$Q_1 = \begin{bmatrix} 0 & Q_{oc} \\ Q_{co} & 0 \end{bmatrix}.$$

The process $\{X(t);\ t \geq 0\}$ will possess an equilibrium distribution, $\boldsymbol{\pi} = (\pi_1, \pi_2, \ldots, \pi_n)^T$ say, where T denotes the transpose, which can be determined from the detailed balance conditions

$$\pi_i q_{ij} = \pi_j q_{ji} \quad (i, j = 1, 2, \ldots, n).$$

We incorporate time interval omission as follows. We assume that a sojourn in the open (*closed*) states is detected if, and only if, it is greater than some critical length τ_0. Suppose that the channel, described by the process $\{X(t); t \geq 0\}$, is detected as being in an open state at time $t = 0$. We assume that the channel continues to be detected as being in an open state until there has been a sojourn of length τ_0 in the closed states, at which point the channel becomes detected as being in a closed state. The channel will remain as such until there has been a sojourn of length τ_0 in the open states, at which point the channel is detected once again as being in an open state, and so on. Set $T'_0 = 0$ and let $T'_1, T'_2, \ldots$ be the times at which channel openings or closings are detected. Let

$$J_k = X(T'_k) \quad (k = 0, 1, \ldots)$$

$$T_k = T'_k - T'_{k-1} \quad (k = 1, 2, \ldots).$$

Set $T_0 = 0$. The Markov process $\{(J_k, T_k); k = 0, 1, \ldots\}$, whose properties are discussed in some detail in Ball and Sansom (1988*a*, *b*), more than describes the observed single-channel record.

The Markov chain $\{J_k; k = 0, 1, \ldots\}$ has transition matrix, P^J say, given by

$$P^J = -[Q_0 - Q_1 Q_0^{-1}\{I - \exp(\tau_0 Q_0)\} Q_1]^{-1} \times Q_1 \exp(\tau_0 Q_0), \quad (12)$$

where $\exp(\tau_0 Q_0) = \Sigma_{k=0}^{\infty} \tau_0^k Q_0^k / k!$ is the usual matrix exponential (see eg. Bellman, 1960). Partition P^J into

$$P^J = \begin{bmatrix} 0 & P^J_{oc} \\ P^J_{co} & 0 \end{bmatrix}.$$

The Markov chain $\{J_{2k}, k = 0, 1, \ldots\}$, which we term the open entry process since it records the state the channel is in every time an opening is detected, has transition matrix P^J_o say, given by

$$P^J_o = P^J_{oc} P^J_{co}. \quad (13)$$

Similarly, the closed entry process $\{J_{2k+1}; k = 0, 1, \ldots\}$ has transition matrix, P^J_c say, given by

$$P^J_c = P^J_{co} P^J_{oc}. \quad (14)$$

The open and closed entry processes possess equilibrium distributions, $\boldsymbol{\pi}_o = (\pi^o_1, \pi^o_2, \ldots, \pi^o_{n_o})^T$ and $\boldsymbol{\pi}_c = (\pi^c_{n_o+1}, \pi^c_{n_o+2}, \ldots, \pi^c_n)^T$ say, which may be determined as follows.

Let $R = (r_{ij})$ be the $n \times n$ matrix given by

$$R = \exp(\tau_0 Q_0) Q_1 [I - Q_0^{-1} (\{I - \exp(\tau_0 Q_0)\} Q_1)^2]^{-1} \exp(\tau_0 Q_0)$$

and

$$\eta_i = \sum_{j=1}^{n} r_{ij} \quad (i = 1, 2, \ldots, n).$$

Then

$$\pi^o_i = \alpha \pi_i \eta_i \quad (i \in O)$$

and

$$\pi^c_j = \beta \pi_j \eta_j \quad (j \in C),$$

where α and β are normalising constants. Recall that π_i is the equilibrium probability that the channel is in state i.

In order to determine dwell time cross-correlations we need expressions for the first and second moments of observed sojourns. Let $F(t)(t \geq 0)$ be the $n \times n$ matrix function defined elementwise by

$$F_{ij}(t) = Pr\{T_k \leq t \text{ and } J_k = j | J_{k-1} = i\} \quad (i, j = 1, 2, \ldots, n).$$

Let $M^{(r)}$ $(r = 1, 2, \ldots)$ be the $n \times n$matrix with elements

$$\mu^{(r)}_{ij} = \int_0^\infty t^r \, dF_{ij}(t) \quad (i, j = 1, 2, \ldots, n).$$

It is shown in Ball and Sansom (1988b) that $M^{(1)}$ and $M^{(2)}$ are given by

$$M^{(1)} = U^{-1}\{(Q_0^{-2} - \tau_0 Q_0^{-1}) Q_1 \exp(\tau_0 Q_0) - V Q_1 P^J\} \quad (15)$$

and

$$\begin{aligned} M^{(2)} = U^{-1}[&-(2Q_0^{-3} - 2\tau_0 Q_0^{-2} + \tau_0^2 Q_0^{-1}) Q_1 \\ &\exp(\tau_0 Q_0) - 2 V Q_1 M^{(1)} \\ &+ \{2Q^{(3)} - \tau_0^2 Q^{(1)} - 2(Q^{(3)} \\ &- \tau_0 Q^{(2)}) \exp(\tau_0 Q_0)\} Q_1 P^J], \end{aligned} \quad (16)$$

where

$$Q^{(1)} = Q_0^{-1} Q_1 Q_0^{-1}$$

$$Q^{(2)} = Q_0^{-2} Q_1 Q_0^{-1} + Q_0^{-1} Q_1 Q_0^{-2}$$

$$Q^{(3)} = Q_0^{-3} Q_1 Q_0^{-1} + Q_0^{-2} Q_1 Q_0^{-2} + Q_0^{-1} Q_1 Q_0^{-3}$$

$$U = I - Q^{(1)}\{I - \exp(\tau_0 Q_0)\} Q_1.$$

and

$$V = (\tau_0 Q^{(1)} - Q^{(2)}) \exp(\tau_0 Q_0) + Q^{(2)}$$

For $r = 1, 2, \ldots$ partition the matrix $M^{(r)}$ into

$$M^{(r)} = \begin{bmatrix} 0 & M^{(r)}_{oc} \\ M^{(r)}_{co} & 0 \end{bmatrix}.$$

Let $\mu^{(r)}_o$ and $\mu^{(r)}_c$ be the unconditional *rth* moments of observed sojourns in the open and closed states, respectively. Then

$$\mu^{(r)}_o = \boldsymbol{\pi}_o^T M^{(r)}_{oc} \mathbf{1} \quad (17)$$

and

$$\mu^{(r)}_c = \boldsymbol{\pi}_c^T M^{(r)}_{co} \mathbf{1}, \quad (18)$$

where $\mathbf{1}$ is the appropriate column vector of ones.

We are now able to determine the theoretical cross-correlation functions. We assume throughout that the observed process is in equilibrium. Recall that the series of single channel open and closed (dwell) times is represented by

$$t_o(1), t_c(1), \ldots, t_o(i), t_o(i), t_c(i), \ldots, t_o(m), t_c(m)$$

which maps into out present notation as

$$T_{2k-1} = t_o(k) \quad k = 1, 2, \ldots$$

$$T_{2k} = t_c(k) \quad k = 1, 2, \ldots.$$

We can then show (see Appendix 1 for proof) that for $k = 0, 1, \ldots$

$$\mathrm{Cov}\,[t_o(i), t_c(i+k)] = \boldsymbol{\pi}_o^T M_{oc}^{(1)} (P_c^J)^k M_{oc}^{(1)} \mathbf{1} - \mu_o^{(1)} \mu_c^{(1)} \quad (19a)$$

and

$$\mathrm{Cov}\,[t_c(i), t_o(i+k+1)\} = \boldsymbol{\pi}_c^T M_{co}^{(1)} (P_o^J)^k M_{oc}^{(1)} \mathbf{1} - \mu_o^{(1)} \mu_c^{(1)} \quad (19b)$$

Now suppose that the transition matrix P_c^J of the closed entry process is diagonisable. Let $\mu_1, \mu_2, \ldots, \mu_{n_c}$ be the eigenvalues of P_c^J with corresponding right eigenvectors $\mathbf{b}_1, \mathbf{b}_2, \ldots, \mathbf{b}_{n_c}$. Let B be the $n_c \times n_c$ matrix whose ith column is $\mathbf{b}_i$ and $C = B^{-1}$. It follows that P_c^J admits the spectral representation

$$P_c^J = \sum_{i=1}^{n_c} \mu_i F_i, \quad (20)$$

where $F_i = \mathbf{b}_i \mathbf{c}_i$, $\mathbf{c}_i$ being the ith row of C.

The matrices $F_1, F_2, \ldots, F_{n_c}$ satisfy

$$F_i F_j = 0 \quad (i \neq j),$$

$$F_i^2 = F_i,$$

and

$$F_1 + F_2 + \ldots + F_{n_c} = I.$$

Furthermore, one of the eigenvalues, μ_1 say, is unity and the remainder have modulus less than one (Cox and Miller, 1965). Also, all the rows of F_1 will be $\boldsymbol{\pi}_c^T$.

Following Fredkin et al. (1985) we define an open gateway state as a channel open state linked directly to one or more closed states, and a closed gateway state as a closed state linked directly to one or more open states. Let N_P be the minimum of the number of open gateway states and closed gateway states (so, for example, $N_P > 1$ for cyclic gating mechanisms with at least two open and two closed states). Then P_c^J has rank at most N_P (Ball and Sansom, 1988*a*), so at most N_P of the μ_i's will be nonzero. Thus Eq. 20 may be written as

$$P_c^J = F_1 + \sum_{i=2}^{N_P} \mu_i F_i, \quad (21)$$

where the summation is omitted if $N_P = 1$. Substituting Eq. 21 into Eq. 19a and noting that the term corresponding to F_1 reduces to $\mu_o^{(1)} \mu_c^{(1)}$ yields

$$\mathrm{Cov}\,[t_o(i), t_c(i+k)] = \boldsymbol{\pi}_o^T M_{oc}^{(1)} \left(\sum_{i=2}^{N_P} \mu_i^k F_i \right) M_{co}^{(1)} \mathbf{1}. \quad (22)$$

Hence the cross-correlation function may be written in the form

$$r_{oc}(k) = \sum_{j=1}^{N_P - 1} \alpha_j \sigma_j^{|k|} (k = 0, 1, \ldots) \quad (23)$$

with $\sigma_j = \mu_{j+1}$ and

$$\alpha_j = \frac{\boldsymbol{\pi}_o^T M_{oc}^{(1)} F_{j+1} M_{co}^{(1)} 1}{(\mathrm{Var}\,[t_o(i)]\ \mathrm{Var}\,[t_c(i)])^{0.5}} \quad (j = 1, 2, \ldots, N_P - 1).$$

Recall from Eqs. 17 and 18 that

$$\mathrm{Var}\,[t_o(i)] = \boldsymbol{\pi}_o^T M_{oc}^{(2)} \mathbf{1} - (\boldsymbol{\pi}_o^T M_{oc}^{(1)} \mathbf{1})^2 \quad (24)$$

and

$$\mathrm{Var}\,[t_c(i)] = \boldsymbol{\pi}_c^T M_{co}^{(2)} \mathbf{1} - (\underline{\pi}_c^T M_{co}^{(1)} \mathbf{1})^2 \quad (25).$$

By a similar treatment, one can show that, provided the transition matrix P_o^J is diagonalisable, the closed to open cross-correlation function may be written in the form

$$r_{co}(k) = \sum_{j=1}^{N_P - 1} \beta_j K_j^{|k|} \quad (k = 0, 1, \ldots). \quad (26)$$

Furthermore, we can prove for cyclic models that

$$\sigma_j = \kappa_j \quad (j = 1, 2, \ldots, N_P - 1)$$

and conjecture that this may generally be the case. Thus both cross-correlation functions are sums of the same geometrically decaying terms (σ_j).

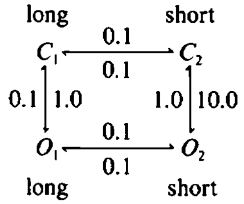
When time interval omission is absent, i.e. $\tau_0 = 0$, several simplifications pertain. Without presenting the details, one can demonstrate that the cross-correlations take the forms in Eqs. 23 and 26, as shown by Colquhoun and Hawkes (1987).

A further special case is that of gating mechanisms for which $N_P = 1$. In this case the matrices P_c^J and P_o^J admit spectral representation as in Eq. 20 whether or not time interval omission is incorporated (Ball and Sansom, 1988b). It follows that all cross-correlations are necessarily null. Thus, experimentally observed nonzero cross-correlations are indicative of a gating mechanism with $N_P > 1$. They cannot be an artifact of time interval omission.

Simple Gating Mechanisms

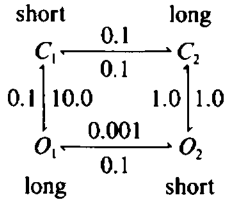
To demonstrate the value of the technique and the way in which cross-correlation functions may be interpreted, we have investigated two simple cyclic ($N_P = 2$) gating mechanisms via simulation studies. The transition rates in these mechanisms have been chosen, rather arbitrarily, to illustrate the features under discussion. In mechanism A the long open state is linked to the long closed state, and the short open state to the short closed state.

Mechanism A



C denotes the closed channel, O the open channel, and the numbers above and below the arrows are the transition rates in units of ms^{-1}. In the second mechanism (B), the long open state is linked to the short closed state, and the short open state to the long closed state:

Mechanism B



For both mechanisms $N_P = 2$, and the horizontal steps (closed-closed and open-open transitions) are relatively slow. Consequently nonzero dwell time autocorrelation functions are seen (results not shown). The use of cross-correlation functions makes it possible to distinguish between these two closely related mechanisms. Mechanism A results in a significant positive cross-correlation between open and closed times (Fig. 1 *a*), whereas mechanism B results in a negative cross-correlation (Fig 1 *b*). That is, for mechanism A a long channel opening is more likely to be preceeded by and succeeded by long channel closings, whereas for mechanism B it is more likely to be preceded by and succeeded by brief closings. Thus, the use of cross-correlation functions allows one to choose between two alternative cyclic gating models for a given set of experimental data.

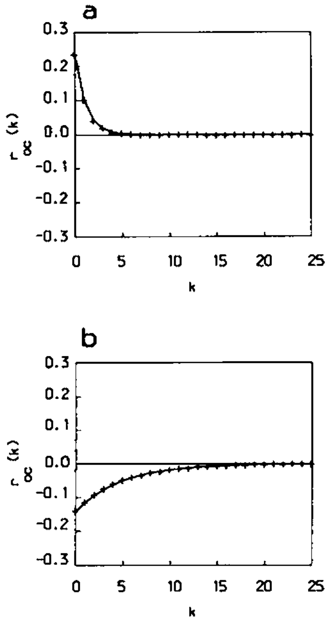
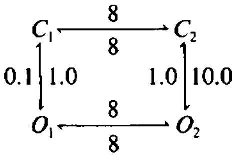


FIGURE 1 Cross-correlation functions for mechanism A (*a*) and mechanism B (*b*). The points represent the sample cross-correlation functions evaluated, using Eq. 2, from simulated data. In each case, 1,000,000 channel events were simulated, with $\tau_0 = 0.00$ ms i.e., no omission of brief events. The solid curves represent the theoretical cross-correlation functions.

Both mechanisms A and B generate what might be described as state switching or gear-shifting (Moczydlowski, 1986) behavior of the channel, e.g. for mechanism A the channel will show an extended series of long openings paired with long closings, and then will switch to an extended period of brief closings paired with brief openings. Such behavior results from the slow (timescale of ~10 ms) interconversion between O_1 and O_2, and correspondingly slow interconversion between C_1 and C_2. We have investigated how increasing the rate of such interconversions, which would be expected to smear out the state-switching, effects the open-closed cross correlation functions. For example, using a more general version of mechanism A.

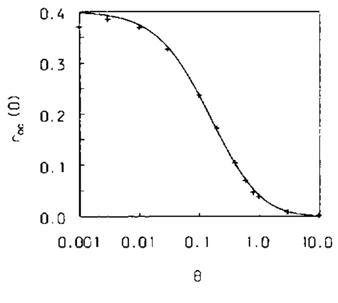
Mechanism A′



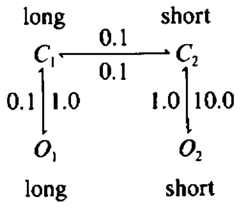
The cross-correlation functions have been evaluated while varying the interconversion rate θ between 0.001 ms^{-1} and 10 ms^{-1}. The results are shown in Fig. 2 where the correlation between an opening and the following closing, $r_{oc}(0)$ is given as a function of θ. It can be seen that for $\theta \leq 0.01\ \mathrm{ms}^{-1}$, $r_{oc}(0)$ plateaus at a limiting value of ~0.40. As θ is increased above 0.01 ms^{-1}, $r_{oc}(0)$ decreases until, at $\theta \approx 1\ \mathrm{ms}^{-1}$, the degree of cross-correlation becomes sufficiently weak that it would be difficult to detect experimentally. This arises because, for $\theta = 1\ \mathrm{ms}^{-1}$, the rates of closed-closed and of open-open interconversions become comparable to the rates of channel opening and closing. Thus, if the channel opens to state O_1 say, then the probabilities of it closing to states C_1 and to C_2 are of similar magnitude. Consequently, correlations between channel open and closed times disappear.

We have also looked at noncyclic variants of mechanisms A and B, in which the two open states lie either side of the closed states, thus still giving $N_P = 2$.

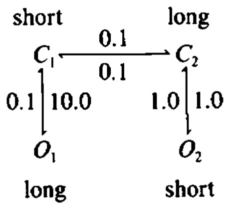
FIGURE 2 Correlation between an opening and the following closing ($r_{oc}(0)$) for mechanism A′ as a function of the interconversion rate θ (see text for details). The points represent the results of simulations of 10,000 events, with no time interval omission. The solid curve represents the corresponding theoretical values.



Mechanism A″



Mechanism B′

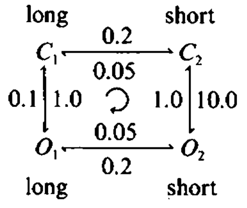


Both mechanisms resulted in significant cross-correlation functions, positive for mechanism A″ ($r_{oc}(0) \approx +0.38$) and negative for mechanism B′($r_{oc}(0) \approx -0.16$). Note that $r_{oc}(0)$ for mechanism A″ is comparable with that for mechanism A′ when $\theta < 0.01$ ms^{-1}. We therefore conclude that cross-correlation functions may also be used to distinguish betweeen noncyclic $N_P = 2$ gating models.

Irreversiblity.

As noted above, irreversiblity of the dwell time series corresponds to a gating mechanism which is not at thermodynamic equilibrium, i.e., which is coupled to an irreversible process, such as movement of ions down an electrochemical gradient (Lauger, 1983; Finkelstein and Peskin, 1984). We have investigated the effect of irreversiblity on dwell time cross-correlation functions using the following mechanism.

Mechanism C



The curved arrow indicates the general direction of flow of the irreversible process. As can be seen from Fig. 3, for mechanism A, which is at thermodynamic equilibrium, $r_{oc} = \{r_{oc}(k), k = 0, 1, \ldots\}$ and $r_{co} = \{r_{co}(k), k = 0, 1, \ldots\}$ are identical, but for mechanism C, which is not at thermodynamic equilibrium, they are distinct. This suggests that inequality of r_{oc} and r_{co} may be used as an indication of irreversibility in the channel gating process. This result still holds in the presence of time interval omission.

The use of cross-correlation functions to test for time irreversiblity has been explored in more detail using a general version of mechanism C.

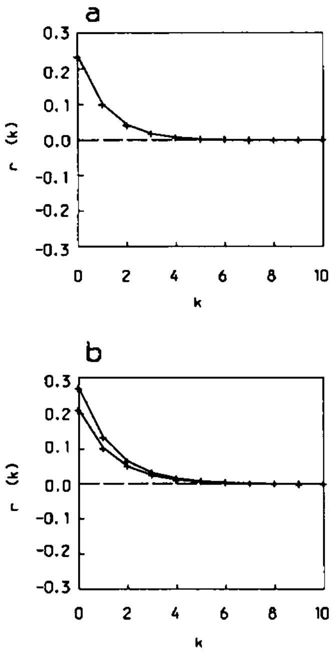
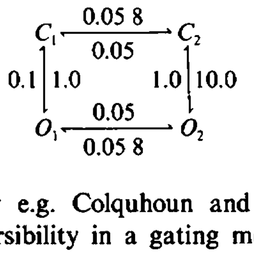


FIGURE 3 Cross-correlation functions (r_{oc} and r_{co}) for mechanism A (*a*) and mechanism C (*b*). The points represent sample and the curves theoretical cross-correlations (see Fig. 1 for details). In (*a*) $r_{oc} = r_{co}$, indicative of a time reversible process. In (*b*) $r_{oc} \neq r_{co}$, corresponding to an irreversible process. The upper line is r_{oc} and the lower line r_{co}.

Mechanism C′



As explained by e.g. Colquhoun and Hawkes (1983) microscopic reversibility in a gating mechanism implies that the product of the clockwise transition rates for a cycle (Π_c say) is equal to the product of the anticlockwise rates (Π_a). One may therefore define the degree of irreversibility in the present gating mechanism in terms of the ratio Π_c/Π_a. For mechanism C, $\Pi_c/\Pi_a = 16$, and for mechanism C′ $\Pi_c/\Pi_a = \theta^2$. The transition matrices P_o^J and P_c^J of the open and closed entry processes for the cyclic mechanism C′ contain the same set of eigenvalues (Ball and Sansom, 1988*a*). It follows from Eqs. 23 and 26, with $N_P = 2$, that the cross-correlation functions take the forms

$$r_{oc}(k) = \alpha_1\sigma_1^k \quad (k = 0, 1, \ldots)$$

and

$$r_{co}(k) = \beta_1\sigma_1^k \quad (k = 0, 1, \ldots).$$

Thus $r_{oc}(k)/r_{co}(k) = \alpha_1/\beta_1$ ($k = 0, 1, \ldots$) and we may take $r_{oc}(0)/r_{co}(0)$ as a measure of the discrepancy between the open-closed and closed-open cross-correlations. We have examined, again by both simulation and calculation, the cross-correlation functions of mechanism C′ as a function of θ using the above measure of the discrepancy. The results of these investigations for θ in the range 1.0 (giving

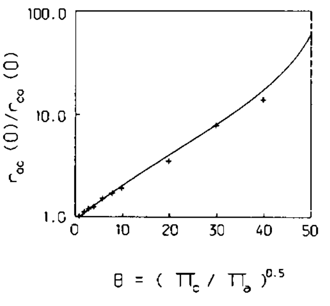
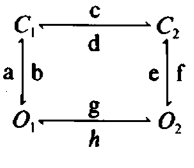


FIGURE 4 The ratio of open-closed vs. closed-open correlations ($r_{oc}(0)/r_{co}(0)$), shown on a logarithmic scale as a function of θ for mechanism C′ (see text for details). The points represent the results of simulations of 10,000 events, with no time interval omission. The solid curve represents the corresponding theoretical values.

$\Pi_c/\Pi_a = 1.0$, i.e., reversible) to 50.0 ($\Pi_c/\Pi_a = 2{,}500$ i.e., markedly irreversible) are presented in Fig. 4. It can be seen that $r_{oc}(0)/r_{co}(0)$, for this mechanism, increases as a function of θ, in an approximately exponential manner. It is reasonable to assume that experimentally one could detect an $r_{oc}(0)/r_{co}(0)$ ratio in excess of 1.2. This would enable one to detect degrees of irreversibility in excess of $\Pi_c/\Pi_a = 10.0$. Of course, a lesser degree of irreversiblity might be detectable if more accurate estimates of the cross correlations could be obtained e.g., by analysis of a greater number of single channel events. Therefore, failure to detect a difference between $r_{oc}(0)$ and $r_{co}(0)$ implies, in this particular case, that the degree of irreversibility is not $>\Pi_c/\Pi_a = 10.0$.

Theory for Simple Gating Mechanisms

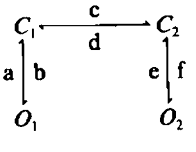
It is possible to use the framework developed earlier to determine theoretical cross-correlation functions for the general 4-state cyclic model.



However, the resulting expressions for the cross-correlation functions are so lengthy that it is difficult to draw general conclusions. We thus have restricted our attention to the detailed numerical studies presented above.

The situation is different when we consider mechanisms A′ and B′, which are both special cases of the following mechanism.

Mechanism D

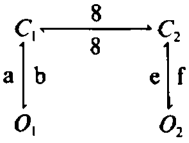


It is shown in Appendix 2 that the signs of all the cross-correlations for a given mechanism are the same. Furthermore, it is shown that, for example:

$$r_{oc}(k)\begin{cases} >0 & \text{if } (a-e)(b-f)>0 \\ =0 & \text{if } (a-e)(b-f)=0 \\ <0 & \text{if } (a-e)(b-f)<0. \end{cases}$$

Thus if we classify the states as being long or short, according to the relative sizes of their opening (closing) rates, the conclusions drawn via examination of mechanisms A′ and B′ are seen to hold generally.

Mechanism D′



It is shown in Appendix 2 that

$$r_{oc}(k) = (b-f)(a-e) \cdot\left[\frac{bf}{\{(be+af)^2+2bf(a-e)^2\}\{4[(b+f)\theta-bf]+2(b+f)^2\}}\right]^{1/2} \cdot\left[\frac{bf}{bf+\theta(b+f)}\right]^{k+1/2} \quad (k=0,1,\ldots). \tag{27}$$

Note that for each $k = 0, 1, \ldots,$ the absolute size of the cross-correlation $r_{oc}(k)$ decreases with increasing θ, tending to zero as θ tends to infinity. Further, the maximum size of $r_{oc}(k)$ is obtained when $\theta = 0$ and is given by

$$\left[\frac{bf}{2\{(be+af)^2+2bf(a-e)^2\}(b^2+f^2)}\right]^{1/2}(b-f)(a-e),$$

(cf. Colquhoun and Hawkes, 1987). Note that the maximal cross-correlation is independent of the lag k. This is because as θ tends to zero the process essentially becomes locked into either the states O_1 and C_1, or the states O_2 and C_2.

GluR Data

Single-channel data collected from the locust GluR in the presence of 10^{-4} M L-glutamate were subjected to cross-correlation analysis. Previous studies (Kerry et al., 1987, 1988) have shown that there is a significant autocorrelation for both channel open and for channel closed times, and have suggested the existence of a weak, negative cross-correlation (Ashford et al., 1984). The cross-correlation results (Fig. 5) reveal a clear negative correlation between channel open times and preceeding and succeeding closed times, extending out to a lag of ~ $k = 10$. This is related to the early observation of Patlak et al. (1979) that the GluR state-switches between a predominantly closed state with brief openings, and a predominantly open state with brief closings.

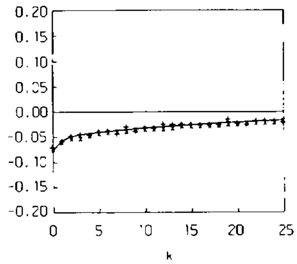


FIGURE 5 Cross-correlation function derived from GluR single-channel data obtained in the presence of 10^{-4}M L-glutamate. Both r_{oc} (+) and r_{co} (x) are shown. The dataset consisted of 50,600 single-channel events recorded from six different membrane sites. The smooth curve represents the fit of the following function to the data $r_{oc}(k) = \alpha_1\sigma_1^{|k|} + \alpha_2\sigma_2^{|k|}$ where $\alpha_1 = -0.026$, $\alpha_2 = -0.050$, $\sigma_1 = 0.32$, and $\sigma_2 = 0.96$.

The cross-correlation function (r_{oc}) for the GluR data has been fitted to Eq. 12 using two components, i.e., $N_P \geq 3$ (Fig. 5). This is in agreement with the results of Kerry et al. (1987), where two components were required to fit the comparable autocorrelation functions.

The data in Fig. 5 are consistent with Eq. 8, i.e., it appears that $r_{oc} = r_{co}$. This supports the assumption, underlying modeling of GluR gating, that GluR single-channel data is time reversible, i.e., that the gating process is at thermodynamic equilibrium. However, it is difficult to be more precise about the degree of irreversibility which would pass unnoticed, as this is almost certainly dependent on the form of the underlying gating mechanism.

A Cooperative Gating Model

We have also investigated dwell time cross-correlation functions for a more complex gating model, which has proved useful in understanding the gating kinetics of the locust GluR (Kerry et al., 1987, 1988; Ball and Sansom, 1988*a*, *b*).

Mechanism E

$$\begin{tikzcd}
C \arrow[r, leftrightarrow] \arrow[d] & CA \arrow[r, leftrightarrow] \arrow[d] & CA_2 \arrow[r, leftrightarrow] \arrow[d] & CA_3 \arrow[r, leftrightarrow] \arrow[d] & CA_4 \arrow[d] \\
O \arrow[r, leftrightarrow] & OA \arrow[r, leftrightarrow] & OA_2 \arrow[r, leftrightarrow] & OA_3 \arrow[r, leftrightarrow] & OA_4
\end{tikzcd}$$

C represents the closed channel, O the open channel and A_n n molecules of agonist bound to the receptor-channel complex. This model is based on the cooperative model for allosteric transitions of proteins of Monod et al. (1965), and the transition matrix used in the present study is given in Table I. The parameter set used is that also employed by Ball and Sansom (1988*a*, *b*), and arose primarily out of simulation studies of GluR gating kinetics. As such, it differs slightly from the experimental estimates presented by Kerry et al. (1988).

Using both simulation studies and theoretical calculations, we examined the cross-correlations which result from adoption of the above gating mechanism (Fig. 6). A negative cross-correlation is seen. Both the correlation for $k = 0$ (i.e., between an opening and the next closing), and the decay of correlation with increasing lag k are comparable to that seen for the GluR data.

TABLE I
TRANSITION MATRIX FOR THE COOPERATIVE GATING MECHANISM

i	j	q_{ij}/ms^{-1}	i	j	q_{ij}/ms^{-1}
1	2	$4ak_{on}^{o}$	6	1	h_1
1	6	h_5/L	6	7	$4ak_{on}^{c}$
2	1	$ak_{on}^{o}/\alpha K_B$	7	2	h_2
2	3	$3ak_{on}^{o}$	7	6	k_{on}^{c}/K_B
2	7	$h_4/\alpha L$	7	8	$3ak_{on}^{c}$
3	2	$2ak_{on}^{o}/\alpha K_B$	8	3	h_3
3	4	$2ak_{on}^{o}$	8	7	$2k_{on}^{c}/K_B$
3	8	$h_3/\alpha^2 L$	8	9	$2ak_{on}^{c}$
4	3	$3ak_{on}^{o}/\alpha K_B$	9	4	h_4
4	5	ak_{on}^{o}	9	8	$3k_{on}^{c}/K_B$
4	9	$h_4/\alpha^3 L$	9	10	ak_{on}^{c}
5	4	$4ak_{on}^{o}/\alpha K_B$	10	5	h_5
5	10	$h_5/\alpha^4 L$	10	9	$4k_{on}^{c}/K_B$

The channel states are numbered $O = 1, \ldots, OA_4 = 5$ for the open states, and $C = 6, \ldots, CA_4 = 10$ for the closed states. The nonzero off-diagonal elements of the transition matrix Q are given above, with the diagonal elements being defined by: $-q_{ii} = \Sigma_{j \neq i} q_{ij}$. Thus q_{ij} is the transition rate for step $i \rightarrow j$ of the gating mechanism. The transition rates are defined in terms of the equilibrium parameters of the model, $\alpha = 10$, $K_B = 2 \times 10^3$ M^{-1}, and $L = 6 \times 10^{-4}$; the closed to open isomerisation rates, $h_1 = 10^{-2}$ ms^{-1}, $h_2 = 1.5 \times 10^{-2}\ \mathrm{ms}^{-1}$, $h_3 = 2.6 \times 10^{-2}\ \mathrm{ms}^{-1}$, $h_4 = 6.7 \times 10^{-1}\ \mathrm{ms}^{-1}$, and $h_5 = 1.7 \times 10^{-1}\ \mathrm{ms}^{-1}$; and the agonist association rates for the closed, $k_{on}^{c} = 10\ \mathrm{ms}^{-1}\ \mathrm{M}^{-1}$, and open states, $k_{on}^{o} = 1\ \mathrm{ms}^{-1}\ \mathrm{M}^{-1}$, where $a = 10^{-4}$ M is the agonist concentration. See Kerry et al. (1988) and F.G. Ball, R. McGee, and M.S.P. Sansom, manuscript submitted for publication.

We have also used this mechanism to explore the effects of failure to detect brief channel openings and closings resulting from time interval omission. Experimentally, such omission results from the limited frequency response of the channel recording and detection systems. Cutoffs (τ_0) of 0.05, 0.10, and 0.20 ms were applied. All channel openings or closings briefer then τ_0 are considered to pass undetected. As can be seen from Fig. 6, the main result of time interval omission is to reduce the amplitude of the cross-correlation function. So, a τ_0 value such that many brief events were missed could result in failure to observe a cross-correlation, but would not lead to observation of an artefactual non-null cross-correlation. Furthermore, as shown above, time interval omission does not alter the form of the cross-correlation functions as defined in Eq. 23. Therefore, even in the presence of time interval omission, fitting of Eq. 23 to cross-correlation function data may be used to obtain a lower bound for N_P.

DISCUSSION

We have demonstrated that cross-correlation functions may be readily evaluated from vectors of single-channel dwell times, and that their interpretation is of use in

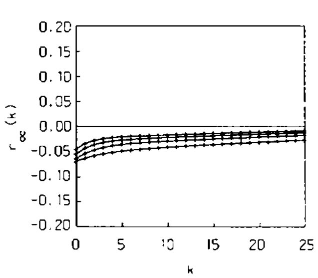


FIGURE 6 Cross-correlation functions derived from the cooperative gating mechanism E (see Table I). The cross-correlations for four values of the cutoff time ($\tau_0 - 0.00$ [*bottom curve*], 0.05, 0.10, and 0.20 [*top curve*] ms) are given. The points correspond to the results of the simulations, each of 3,000,000 events, and the smooth curves are derived from the theory presented in the text, and in Ball and Sansom (1988*a*, *b*). As can be seen, both the magnitude of $r_{oc}(0)$ and the rate of decay of the cross-correlation function is altered by time interval omission. This corresponds to decreases in both α_i and σ_i with increasing τ_0.

distinguishing between alternative cyclic or branched gating mechanisms. Indeed, one might suggest that cross-correlation functions be used in place of autocorrelation functions, as the former convey additional information. Specifically, the observation of a non-null cross-correlation function is indicative of an $N_P > 1$ gating mechanism. Such mechanisms include all cyclic models, and those branched models where there are multiple open and closed gateway states. The sign of the cross-correlation function then provides information concerning the pairing of the open states of the channel with the closed. The sign of the cross-correlation is the same as the sign of the correlation between mean adjacent dwell times in analysis by the method of McManus et al. (1985). We have demonstrated that, for simple cyclic gating mechanisms, the sign of the cross-correlation function may be derived from the values of the transition rates in a relatively straightforward manner.

The studies on the effect of the open-open and closed-closed transition rates have an important implication with respect to the interpretation of observed null cross-correlation functions. Failure to observe cross-correlation may result either from an $N_P = 1$ mechanism, or from an $N_P > 1$ mechanism with relatively high open-open and closed-closed transition rates. Thus, only non-null cross-correlation functions may be unambiguously interpreted.

Cross-correlation analysis may be used as an alternative to the method of Steinberg (1987a) to test whether or not the gating process is at thermodynamic equilibrium. Irreversiblity of channel gating kinetics reveals itself via failure of the equality in Eq. 8. It has been shown that it is possible to estimate an upper bound on the degree of irreversibility (as defined by Π_a/Π_c) for a simple cyclic gating mechanism by evaluation of $r_{oc}(0)/r_{co}(0)$ from the experimental data. However, complications arise when attempting to generalise this approach to more complex models. For example, in Mechanism E there are four cyclic pathways leading away from and back to state C, any of which might be irreversible. This presents difficulties in defining a single measure of the degree of irreversibility analogous to Π_a/Π_c above. Furthermore, the interpretation of an apparent equality of $r_{oc}(0)$ and $r_{co}(0)$ in terms of an upper bound on the degree of irreversibility will be highly dependent on the form of the gating mechanism underlying a given set of experimental single-channel data.

The use of cross-correlation functions may be extended by fitting the sample functions with sums of geometrical decays. As with autocorrelation data, the number of terms fitted provides a lower bound on N_P and hence may aid in model identification from experimental data.

We have also explored the effects of time interval omission on cross-correlation functions. This is important because, as emphasised by Colquhoun and Hawkes (1987), time interval omission may result in a burst of openings appearing as a single opening. Our results suggest that the primary effect of interval omission is to reduce the extent of any cross-correlation between dwell times. This is directly comparable to the effects of event omission on autocorrelation functions, as described by Ball and Sansom (1988*a*, *b*). The outcome of this is that interval omission does not result in spurious cross-correlations, but may lead to failure to detect a correlation. In terms of mechanistic interpretation of channel kinetics, this implies that event omission may result in a bias towards over-simplified gating models.

The cross-correlation analysis of the GluR is compatible with the results of Kerry et al. (1988). Fitting the sample cross-correlation function gives an estimate of $N_P \geq 3$. This minimum estimate of the number of open-closed isomerisation pathways is in agreement with that obtained earlier (Kerry et al., 1987) from analysis of autocorrelation functions. The negative cross-correlation is in agreement with the initial observations of state-switching (Patlak et al., 1979; Gration et al., 1981). Furthermore, cross-correlation analysis supports the assumption that the gating mechanism is at thermodynamic equilibrium, and is not coupled to an irreversible process.

The cooperative model for the GluR presented results in negative cross-correlations comparable to those observed experimentally. This may be readily understood by considering the properties of this model in a little more detail. The nature of the cooperative model is such that the equilibrium:

$$CA_n \underset{h'_{n+1}}{\overset{h_{n+1}}{\rightleftharpoons}} OA_n$$

shifts in favor of OA_n with increasing n (number of bound agonist molecules). So, at low n $h_{n+1} \ll h'_{n+1}$, whereas at high n $h_{n+1} \gg h'_{n+1}$. This means that, at low n, short openings are paired with long closings, and that at high n, long openings are paired with short closings. So, given the relatively slow agonist dissociation and association rates in this model, negative cross-correlations arise. Indeed, this is one of the reasons that this class of gating mechanisms has been adopted for further investigation in attempting to understand the gating kinetics of the GluR (Kerry et al., 1987, 1988). It is worth noting that such models, in general, are capable of explaining the gearshift phenomenon described by Moczydlowski (1986) with particular reference to batrachotoxin-activated Na Channels.

In summary, it can be seen that cross-correlations are an easy-to-implement and useful addition to the statistical tools for examination of single-channel kinetics.

APPENDIX I

Proof of Eqs. 19a and 19b.

We prove the formula for Cov $[t_o(i), t_c(i+k)]$; that for Cov $[t_c(i), t_o(i+k+1)]$ is proved similarly. For $j \epsilon C$ let

$$I\{J_1 = j\} = \begin{cases} 1 & \text{if } J_1 = j \\ 0 & \text{otherwise,} \end{cases}$$

be the indicator function of the event $\{J_1 = j\}$. Then, for $k = 0, 1, \ldots,$

$$E[t_o(1)t_c(1+k)] = E_{J_o}[E[t_o(1)t_c(1+k)|J_0 = i]]$$

$$= E_{J_o}\left[E\left[\sum_{j\epsilon C} t_o(1)t_c(1+k)I\{J_1 = j\}|J_0 = i\right]\right]$$

$$= \sum_{j\epsilon C} E_{J_o}[E[t_o(1)t_c(1+k)I\{J_1 = j\}|J_0 = i]]$$

$$= \sum_{j\epsilon C} E_{J_o}[E[t_o(1)I\{J_1 = j\}|J_0 = i]]E[t_c(1+k)|J_1 = j],$$

since the embedded process $\{(J_k, T_k);\ k = 0, 1, \ldots,\}$ is Markov. Conditioning on J_{2k+1}, the entry state of the sojourn $t_c(1+k)$, we have

$$E[t_c(1+k)|J_1 = j]$$
$$= E_{J_{2k+1}|J_1=j}[E[t_c(1+k)|J_{2k+1} = l]] = [(P_c^J)M_{co}^{(1)}I]_j,$$

since $\{J_k;\ k = 0, 1, \ldots\}$ is Markov. It follows that

$$E[t_o(1)t_c(1+k)]$$

$$= \sum_{j\epsilon C} E_{J_o}[E\{t_o(1)I\{J_1 = j\}|J_0 = i]][(P_c^J)^k M_{co}^{(1)}\mathbf{1}]_j$$

$$= \sum_{j\epsilon C}\sum_{i\epsilon O} \pi_i^o \mu_{ij}^{(1)}[(\mathrm{P}_c^J)^k M_{co}^{(1)}\mathbf{1}]_j$$

$$= \boldsymbol{\pi}_o^T M_{oc}^{(1)}(P_c^J)^k M_{co}^{(1)}\mathbf{1}.$$

Hence, by the stationarity of $(t_o(1), t_c(1))$, $(t_o(2), t_c(2)), \ldots,$

$$\mathrm{Cov}\,[t_o(i), t_c(i+k)] = \boldsymbol{\pi}_o^T M_{oc}^{(1)}(P_c^J)^k M_{co}^{(1)}\mathbf{1} - \mu_o^{(1)}\mu_c^{(1)}$$

as required (Eq. 19a).

APPENDIX 2

Derivation of Cross-Correlation Functions for Mechanisms D and D′

We present the derivation of the cross-correlation functions for mechanisms D and D′, when there is no time interval omission. Derivations incorporating time interval omission are similar, but with more complicated algebra. Mechanisms D and D′ are time reversible, since their graphs are trees, so $r_{oc}(k) = r_{co}(k)$ for all k. Thus we need only determine $r_{oc}(k)$, say.

Label the channel states O_1, O_2, C_1, C_2 by 1, 2, 3, 4 respectively. Our starting point is the transition rate matrix Q given by:

$$Q = \begin{bmatrix} Q_{oo} & Q_{oc} \\ Q_{co} & Q_{cc} \end{bmatrix}$$
$$= \begin{bmatrix} -a & 0 & a & 0 \\ 0 & -e & 0 & e \\ b & 0 & -(b+c) & c \\ 0 & f & d & -(f+d) \end{bmatrix}.$$

The transition matrices, P_o^J and P_c^J, of the open and closed entry processes are given by Eqs. 13 and 14, after substitution from Eq. 12 with $\tau_0 = 0$. We find that $P_o^J = P_c^J$ and

$$P_o^J = (bf + bd + cf)^{-1}\begin{bmatrix} bd + bf & cf \\ bd & bf + cf \end{bmatrix}.$$

The equilibrium distributions of the open and closed entry processes, $\boldsymbol{\pi}_o$ and $\boldsymbol{\pi}_c$ are also equal. For example, $\boldsymbol{\pi}_o$ satisfies $\boldsymbol{\pi}_o^T P_o^J = \boldsymbol{\pi}_o^T$ and $\boldsymbol{\pi}_o^T\mathbf{1} = 1$, from which we obtain $\pi_1^o = bd(bd + cf)^{-1}$ and $\pi_2^o = cf(bd + cf)^{-1}$. The mean matrices $M_{oc}^{(1)}$ and $M_{co}^{(1)}$, which may be derived from Eq. 15 with $\tau_0 = 0$, are:

$$M_{oc}^{(1)} = \begin{bmatrix} a^{-1} & 0 \\ 0 & e^{-1} \end{bmatrix}$$

and

$$M_{co}^{(1)} =$$
$$(bf + bd + cf)^{-2}\begin{bmatrix} (d+f)^2 b + bcd & cf(b+c+d+f) \\ bd(b+c+d+f) & (b+c)^2 f + cdf \end{bmatrix}.$$

In order to express $r_{oc}(k)$ in the form of Eq. 23, we require the spectral representation of P_o^J (cf., Eq. 20). After some algebra we obtain eigenvalues $\mu_1 = 1$ and $\mu_2 = bf/(bf + bd + cf)$, with the corresponding matrix terms

$$F_1 = (bd + cf)^{-1}\begin{bmatrix} bd & cf \\ bd & cf \end{bmatrix},$$

and

$$F_2 = (bd + cf)^{-1} \begin{bmatrix} cf & -cf \\ -bd & bd \end{bmatrix}.$$

It then follows from Eq. 22 that

$$\mathrm{Cov}\,[t_o(i), t_c(i + k)] = \frac{(b - f)(a - e)bcdf}{(bd + cf)^2(bf + bd + cf)ae} \cdot \left[\frac{bf}{bf + bd + cf}\right]^k \quad (k = 0, 1, \ldots) \qquad \text{(A1)}$$

In order to determine the cross-correlation function $r_{oc}(k)$ we need to find Var $[t_o(i)]$ and Var $[t_c(i)]$. From Eqs. 12 and 18 we can show that:

$$\mu_o^{(1)} = (bd + cf)^{-1}[(bda^{-1}) + (cfe^{-1})]$$

and

$$\mu_c^{(1)} = (bd + cf)^{-1}(c + d).$$

Also, using Eq. 16 with $\tau_0 = 0$ we can show that

$$M_{oc}^{(2)} = 2\begin{bmatrix} a^{-2} & 0 \\ 0 & e^{-2} \end{bmatrix},$$

and substitution into Eq. 24 yields

$$\mathrm{Var}\,[t_o(i)] = (bd + cf)^{-2}[\{(bda^{-1}) + (cfe^{-1})\}^2 + 2bcdf\{(a^{-1} - e^{-1})\}^2]. \qquad \text{(A2)}$$

Similarly,

$$\mathrm{Var}\,[t_c(i)] = \frac{(c + d)^2(bd + cf - bf) + 2(bc + df)(bd + cf)}{(bf + bd + cf)(bd + cf)^2}, \qquad \text{(A3)}$$

and hence $r_{oc}(k)$ is now determined (cf., Eq. 1).

Turning now to mechanism D′, we have $c = d = \theta$. It follows from Eq. A1 that

$$\mathrm{Cov}\,[t_o(i), t_c(i)] = \frac{(b - f)(a - e)bf}{ae(b + f)^2(bf + \theta(b + f))}.$$

Also, from Eqs. A2 and A3 we have

$$\mathrm{Var}\,[t_o(i)] = \frac{(be + af)^2 + 2bf(a - e)^2}{(b + f)^2a^2e^2}$$

and

$$\mathrm{Var}\,[t_c(i)] = \frac{4\{(b + f)\theta - bf\} + 2(b + f)}{(bf + \theta(b + f))(b + f)^2}.$$

Now $N_p = 2$, so we have from Eq. 23 that $\alpha_1 = r_{oc}(0)$. Thus $r_{oc}(k)$ is given by Eq. 27.

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