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Relaxation and fluctuations of membrane currents that flow through drug-operated channels

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The theoretical background is presented for (*a*) the relaxation towards equilibrium of drug-induced membrane currents, and (*b*) the fluctuations of membrane current about its equilibrium value that originate in the opening and closing of membrane ion channels. General expressions are given that relate the relaxation current, autocovariance function, spectral density function, fluctuation variance and mean open channel lifetime to the rate constants and single channel conductances for any theory of drug action based on the law of mass action. The question of how much can be validly inferred from experimental spectra that appear to have only one component is discussed. The equations are illustrated by their application to some simple theories of drug action that are currently under consideration.

INTRODUCTION

In the last few years the measurement of stochastic fluctuations in drug-induced membrane currents has produced interesting new information concerning the mechanism of drug action (Katz & Miledi 1970, 1972, 1973, 1975; Stevens 1972; Anderson & Stevens 1973; Crawford & MacBurney 1976; Dreyer, Walther & Peper 1976; Neher & Sakmann 1976*a*; Colquhoun, Dionne, Steinbach & Stevens 1975; Colquhoun, Large & Rang 1977). More recently, similar information has been obtained by measuring the relaxation of the membrane current following a perturbation of the system by means of a step change in membrane potential (Adams 1975*a*; Neher & Sakmann 1975; Sheridan & Lester 1975).

The primary effect of all the drugs under consideration is to cause the opening of ion permeable channels in the endplate membrane of muscle fibres, thus increasing the membrane conductance. Attention will be restricted to the case where the potential difference across the membrane is held constant by means of a voltage clamp circuit, so that the time course of conductance changes is not distorted by the passive electrical properties of the membrane. Furthermore it will be supposed, in common with other workers in the field, that (*a*) the opening and closing of a single channel causes a rectangular pulse of current, of amplitude y, to flow (see Anderson & Stevens 1973; Neher & Sakmann 1976*b*) and (*b*) that the instantaneous current–

voltage relation is linear, i.e. ohmic (Anderson & Stevens 1973; Lester, Changeux & Sheridan 1975). Thus

$$y = \gamma(V - V_{eq}), \tag{1}$$

where γ is the conductance of a single channel, V is the (clamped) membrane potential, and V_{eq} is the equilibrium (zero-current) potential.

The signal produced by a single channel is too small to be observed using conventional techniques (but see Neher & Sakmann 1976*b*). However, the moment-to-moment variation in the number of open channels gives rise to fluctuations about the mean current. Spectral analysis of these fluctuations can give information about the behaviour of single channels (Stevens 1972).

The classical theory of drug action (see, for example, Clark 1933) suggested that when a drug, A, combined with a closed channel T (state 2 say) an active complex R (open channel; state 1 say) was formed,

$$\underset{\text{state 2}}{A + T} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \underset{\text{state 1}}{AR}. \tag{2}$$

However, this simple theory has proved unsatisfactory, for three main reasons. First, it has been found that some drugs (partial agonists) cannot open all channels, even at high drug concentrations, contrary to the predictions of this theory. Such drugs are said to have low *efficacy* (Stephenson 1956). Secondly, the curve of concentration against response does not have the simple hyperbolic shape implied by the simple theory, but is sigmoid, i.e. it shows cooperativity (Katz & Thesleff 1957; Jenkinson 1960; Karlin 1967; Changeux & Podleski 1968; Takeuchi & Takeuchi 1969; Rang 1973; Peper, Dreyer & Müller 1975; Adams 1975*b*, *c*). Thirdly, it provides no physical explanation of why drug binding should cause a channel to open.

For these reasons, and in an attempt to understand the physical (i.e. molecular) mechanisms involved in drug action, a number of more complicated theories have been proposed in recent years (e.g. Karlin 1967; Changeux & Podleski 1968; Colquhoun 1973, 1975; Thron 1973; Rang 1975).

Up to now it has not proved possible to distinguish experimentally between the various mechanisms that have been postulated. However, the observation of two components in the spectral density of the fluctuations about the mean current induced by certain agonists (Colquhoun *et al.* 1975) gives some reason to hope that useful information can be obtained from this sort of experiment. It is the purpose of this paper to provide a general method for calculation of the spectral density, relaxation rate and related quantities, and to exemplify these methods by some of the theories of drug action that are of interest at the moment. Related work has been reported by Stevens (1972), Anderson & Stevens (1973), Hammes & Wu (1974), Verveen & DeFelice (1974), Chen (1975), and Conti & Wanke (1975).

Stochastic formulation of theories

The law of mass action, as it is usually applied to mechanisms of drug action, implies that the lifetime of each chemical species present is a memoryless random variable that does not depend on the age of the species. Therefore future states of the system depend only on its present state, and not on how that state was reached, i.e. the system can be treated as a Markov process with discrete states in continuous time (see, for example, Cox & Miller 1965). In this paper we shall deal only with the case of constant drug concentration, so the probabilities of transition between one state and another may be assumed constant (not time dependent).

The basic expression that defines the system is the probability that a channel will undergo a transition from one state to another during a time interval $\Delta t = \tau$. We define $P_{ij}(\tau)$ as the probability that the system will be in state j at time $t+\tau$, given that it was in state i at time t:

$$P_{ij}(\tau) = \text{prob}[j \text{ at } t+\tau | i \text{ at } t]. \tag{3}$$

The $k \times k$ matrix with elements $P_{ij}(\tau)$ will be denoted $\boldsymbol{P}(\tau)$, where k is the total number of kinetically distinguishable states (see p. 246) that the system can adopt. The P_{ij} are functions of τ, but not of t because of the assumptions above. In the simplest possible case, equation (2) above, there are $k = 2$ states and the transition probabilities are, for closing $(1 \rightarrow 2)$,

$$P_{12}(\tau) = k_2\tau + o(\tau), \tag{4}$$

and for *not* closing (the only other possibility)

$$P_{11}(\tau) = 1 - k_2\tau - o(\tau); \tag{5}$$

for opening $(2 \rightarrow 1)$

$$P_{21}(\tau) = k_1 x_A \tau + o(\tau), \tag{6}$$

and for *not* closing

$$P_{22}(\tau) = 1 - k_1 x_A \tau - o(\tau), \tag{7}$$

where x_A is the concentration of drug, and $o(\tau)$ is a quantity that includes the possibility of more than one transition occurring during the interval τ, which becomes negligible when τ is short,

$$\lim_{\tau \to 0} \left(\frac{o(\tau)}{\tau} \right) = 0. \tag{8}$$

An elementary account of transition probabilities of this sort is given by Colquhoun (1971, chap. 5 and appendix 2). For a more rigorous account see, for example, Cox & Miller (1965).

The usual basic equation is found by differentiating $\boldsymbol{P}(t)$ (see Cox & Miller (1965), pp. 178–182; cf. Colquhoun (1971), pp. 375–376, 380–385). This gives

$$\mathrm{d}\boldsymbol{P}(t)/\mathrm{d}t = \boldsymbol{P}(t)\,\boldsymbol{Q}, \tag{9}$$

which has the formal solution (because $\boldsymbol{P}(0) = \boldsymbol{I}$)

$$\boldsymbol{P}(t) = \mathrm{e}^{\boldsymbol{Q}t}, \tag{10}$$

where $\boldsymbol{Q}$ is a matrix of *constants* defined as

$$\boldsymbol{Q} = \lim_{\tau \to 0} \left[\frac{\boldsymbol{P}(\tau) - \boldsymbol{I}}{\tau} \right], \tag{11}$$

where $\boldsymbol{I}$ is the unit matrix. In other words, elements of $\boldsymbol{Q}$ are found by ignoring the $o(\tau)$ terms and taking P_{ij}/τ for the off-diagonal elements ($i \neq j$) and $(P_{ij}-1)/\tau$ for the diagonal elements ($i = j$).

Thus, in the simple example above we have

$$\boldsymbol{Q} = \begin{bmatrix} -k_2 & k_2 \\ k_1 x_A & -k_1 x_A \end{bmatrix}. \tag{12}$$

It is true in general, as in this case, that the rows of $\boldsymbol{P}$ sum to unity (i.e. the system *must* be in one of its possible states), and that the rows of $\boldsymbol{Q}$ sum to zero, so $\boldsymbol{Q}$ is singular.

The following results, which are well known, will be given now for reference later. Let $\lambda_1, \lambda_2, \ldots, \lambda_k$ denote the eigenvalues of $\boldsymbol{Q}$. One of these, λ_1, say, must be zero as $\boldsymbol{Q}$ is singular. Many library computer programs are available for finding the eigenvalues and eigenvectors of a matrix, though numerical difficulties may be encountered in particular cases. We shall deal here only with the case where all the eigenvalues are different. In this case we may express $\boldsymbol{Q}$ in the form

$$\boldsymbol{Q} = \boldsymbol{M}\boldsymbol{\Lambda}\boldsymbol{N}, \tag{13}$$

where $\boldsymbol{\Lambda} = \text{diag}(\lambda_1, \ldots, \lambda_k)$, $\boldsymbol{M}$ is a matrix the k columns ($\boldsymbol{m}_j$) of which are the k eigenvectors of $\boldsymbol{Q}$, and $\boldsymbol{N} = \boldsymbol{M}^{-1}$. If we denote the ith row of $\boldsymbol{N}$ as $\boldsymbol{n}_i$, then we can define the spectral expansion of $\boldsymbol{Q}$,

$$\boldsymbol{Q} = \sum_{i=1}^{k} \boldsymbol{A}_i \lambda_i, \tag{14}$$

where

$$\boldsymbol{A}_i = \boldsymbol{m}_i \boldsymbol{n}_i. \tag{15}$$

The matrices, $\boldsymbol{A}_i$, have the following properties:

$$\left.\begin{aligned} \boldsymbol{A}_i \boldsymbol{A}_j &= \boldsymbol{0} \quad (i \neq j); \\ \boldsymbol{A}_i \boldsymbol{A}_i &= \boldsymbol{A}_i; \end{aligned}\right\} \tag{16}$$

$$\sum_{i=1}^{k} \boldsymbol{A}_i = \boldsymbol{I}. \tag{17}$$

By the use of these relations, the solution, (10), can be put into the form

$$\boldsymbol{P}(t) = \sum_{i=1}^{k} \boldsymbol{A}_i e^{\lambda_i t}. \tag{18}$$

Let $p_j(t)$ be the probability that a channel is in state j at time t. It is true in general (see the examples following (25) and (83) for particular cases) that the first column

of $\boldsymbol{M}$ may be given elements that are all unity; that the first row of $\boldsymbol{N}$ consists of the equilibrium occupancies, $p_j(\infty)$; and that the jth column of $\boldsymbol{A}_1$ therefore has all elements equal to $p_j(\infty)$. Thus if an element of $\boldsymbol{A}_m$ is denoted $a_{ij}^{(m)}$,

$$n_{1j} = a_{ij}^{(1)} = p_j(\infty), \tag{19}$$

and hence, from (17),

$$\sum_{m=2}^{k} a_{ij}^{(m)} = \delta_{ij} - p_j(\infty), \tag{20}$$

where $\delta_{ij} = 1$ if $i = j$, and $\delta_{ij} = 0$ otherwise.

Relaxation after perturbation

Let $\boldsymbol{p}(t)$ be the $1 \times k$ row vector with elements $p_j(t)$. Now

$$p_j(t) = \sum_{i=1}^{k} p_i(0)\, P_{ij}(t), \quad \text{where} \quad j = 1, 2, \ldots, k, \tag{21}$$

or, in matrix form,

$$\boldsymbol{p}(t) = \boldsymbol{p}(0)\, \boldsymbol{P}(t). \tag{22}$$

(This could also be written as $\boldsymbol{p}(t+\tau) = \boldsymbol{p}(t)\, \boldsymbol{P}(\tau)$, so $\mathrm{d}\boldsymbol{p}(t)/\mathrm{d}t = \boldsymbol{p}(t)\, \boldsymbol{Q}$, where $\boldsymbol{Q}$ is as defined in (11); a simple example is given in Colquhoun (1971), p. 381.)

The solution of equation (22), from (10) and (18), can be written

$$\boldsymbol{p}(t) = \boldsymbol{p}(0)\, \mathrm{e}^{\boldsymbol{Q}t} = \boldsymbol{p}(0) \sum_{i=1}^{k} \boldsymbol{A}_i\, \mathrm{e}^{\lambda_i t} \tag{23}$$

$$= \boldsymbol{p}(\infty) + \boldsymbol{p}(0) \sum_{i=2}^{k} \boldsymbol{A}_i\, \mathrm{e}^{\lambda_i t}, \tag{24}$$

where $\boldsymbol{p}(0)$ defines the state of the system before the perturbation is applied. The second form follows because $\lambda_1 = 0$; $\mathrm{Re}\,(\lambda_i) < 0$ for $i > 1$, so $\boldsymbol{p}(\infty) = \boldsymbol{p}(0)\, \boldsymbol{A}_1$.

The manipulations can be illustrated explicitly for the simple classical model, equation (2). The elements of $\boldsymbol{P}(\tau)$ are given in (4)–(7). $\boldsymbol{Q}$ is given in (12). The eigenvalues of $\boldsymbol{Q}$ are $\lambda_1 = 0$, $\lambda_2 = -(k_1 x_\mathrm{A} + k_2) = -k_2(c+1)$, where the dimensionless normalized concentration, c, is defined as

$$c = x_\mathrm{A}/(k_2/k_1). \tag{25}$$

The eigenvectors of $\boldsymbol{Q}$ are, up to an arbitrary constant,

$$\boldsymbol{m}_1 = \begin{bmatrix} 1 \\ 1 \end{bmatrix}, \quad \boldsymbol{m}_2 = \begin{bmatrix} 1 \\ -c \end{bmatrix},$$

so $\boldsymbol{M} = \begin{bmatrix} 1 & 1 \\ 1 & -c \end{bmatrix}$, $\det(\boldsymbol{M}) = -(c+1)$, and $\boldsymbol{N} = \boldsymbol{M}^{-1} = \begin{bmatrix} \dfrac{c}{c+1} & \dfrac{1}{c+1} \\ \dfrac{1}{c+1} & -\dfrac{1}{c+1} \end{bmatrix}$.

Thus, using (15),

$$\boldsymbol{A}_1 = \begin{bmatrix} \frac{c}{c+1} & \frac{1}{c+1} \\ \frac{c}{c+1} & \frac{1}{c+1} \end{bmatrix}, \quad \boldsymbol{A}_2 = \begin{bmatrix} \frac{1}{c+1} & -\frac{1}{c+1} \\ -\frac{c}{c+1} & \frac{c}{c+1} \end{bmatrix}.$$

It can easily be verified that these obey (16) and (17). Thus, from the fact that $p_1(\infty) = c/(c+1)$, and $p_2(0) = 1 - p_1(0)$, equation (24) gives

$$\begin{aligned} p_1(t) &= p_1(\infty) + \left[p_1(0)\left(\frac{1}{c+1}\right) + p_2(0)\left(\frac{-c}{c+1}\right)\right] e^{\lambda_2 t} \\ &= p_1(\infty) + [p_1(0) - p_1(\infty)]\, e^{-k_2(c+1)t}, \end{aligned}$$

which is the well-known deterministic result first given by Hill (1909).

Use of (20) shows that in *any* case where there are only two kinetically distinguishable states ($k = 2$), $a_{ij}^{(2)} = \delta_{ij} - p_j(\infty)$ so (24) gives the relaxation as

$$p_1(t) = p_1(\infty) + [p_1(0) - p_1(\infty)]\, e^{\lambda_2 t}. \tag{26}$$

Relaxation of membrane current

Suppose, in general, that r states correspond to open channels, with conductance γ_i, and that $k-r$ states correspond with closed channels, with $\gamma_i = 0$. The expected total current flowing at time t, $I(t)$, will therefore be, from (1),

$$\begin{aligned} I(t) &= N(V - V_{eq}) \sum_{i=1}^{k} p_i(t)\, \gamma_i \\ &= N(V - V_{eq})\, \boldsymbol{p}(0)\, e^{\boldsymbol{Q}t}\, \boldsymbol{\Gamma u}, \end{aligned} \tag{27}$$

where N is the total number of channels, and $p_i(t)$ is given by (24).

If (24) is substituted into (27) the result can be written

$$I(t) - I(\infty) = N(V - V_{eq})\, \boldsymbol{p}(0) \sum_{i=2}^{k} \boldsymbol{A}_i\, e^{\lambda_i t}\, \boldsymbol{\Gamma u}, \tag{28}$$

where $\boldsymbol{\Gamma}$ is a diagonal matrix of the channel conductances γ_i ($i = 1, \ldots, k$) and $\boldsymbol{u}$ is a $k \times 1$ vector with unit elements, so

$$I(\infty) = N(V - V_{eq})\, \boldsymbol{p}(0)\, \boldsymbol{A}_1\, \boldsymbol{\Gamma u} = N(V - V_{eq})\, \boldsymbol{p}(\infty)\, \boldsymbol{\Gamma u}.$$

This shows that the current relaxes as the sum of $k-1$ exponential terms, with rate constants $\lambda_2, \lambda_3, \ldots, \lambda_k$. Notice that $\boldsymbol{\Gamma u}$ is just a column vector with elements γ_i; we prefer to use this apparently cumbersome notation because it leads to greater elegance later on.

If the numbering convention is adopted that states $1, 2, \ldots, r$ are open states with conductance γ_i, and the remaining states are shut ($\gamma_i = 0$), then the matrices that have been used can conveniently be partitioned thus

$$\boldsymbol{P} = \left[\begin{array}{c:c} \boldsymbol{P}_{RR} & \boldsymbol{P}_{RT} \\ \hdashline \boldsymbol{P}_{TR} & \boldsymbol{P}_{TT} \end{array}\right], \quad \boldsymbol{Q} = \left[\begin{array}{c:c} \boldsymbol{Q}_{RR} & \boldsymbol{Q}_{RT} \\ \hdashline \boldsymbol{Q}_{TR} & \boldsymbol{Q}_{TT} \end{array}\right]. \tag{29}$$

So $\boldsymbol{P}_{RR}$, the top left-hand $r \times r$ elements of $\boldsymbol{P}$, refers to transitions between open states. Likewise define

$$\boldsymbol{p}(\infty) = [\boldsymbol{p}_R(\infty) \vdots \boldsymbol{p}_T(\infty)], \quad \boldsymbol{\Gamma} = \left[\begin{array}{c:c} \boldsymbol{\Gamma}_R & \boldsymbol{0} \\ \hdashline \boldsymbol{0} & \boldsymbol{0} \end{array}\right]. \tag{30}$$

Most of the results given here are correct when written with the original unpartitioned matrices (and no numbering convention) but it is more economical, both for algebra and for computing, to use the partitioned forms.

With this notation, (28) can be written as

$$I(t) - I(\infty) = N(V - V_{eq})[\boldsymbol{p}_R(0)\boldsymbol{X}_{RR} + \boldsymbol{p}_T(0)\boldsymbol{X}_{TR}]\boldsymbol{\Gamma}_R\boldsymbol{u}_R, \tag{31}$$

where $\boldsymbol{X} = \sum_2^k \boldsymbol{A}_i e^{\lambda_i t}$ is partitioned as in (29), and $\boldsymbol{u}_R$ is an $r \times 1$ vector with unit elements. Alternatively this can be written explicitly as the sum of exponential terms

$$I(t) - I(\infty) = N(V - V_{eq}) \sum_{m=2}^{k} b_m e^{\lambda_m t}, \tag{32}$$

where the coefficients are

$$b_m = \sum_{i=1}^{k} \sum_{j=1}^{r} p_i(0)\gamma_j a_{ij}^{(m)}. \tag{33}$$

If there are $k = 2$ states only, one of which (state 1) is open (with conductance γ) and the other shut, then, from (20) and (28), (31) or (32), the result is the well-known expression

$$\begin{aligned} I(t) - I(\infty) &= N(V - V_{eq})\gamma[p_1(0) - p_1(\infty)]e^{\lambda_2 t} \\ &= [I(0) - I(\infty)]e^{\lambda_2 t}. \end{aligned} \tag{34}$$

Characteristics of current fluctuations

The observed membrane current will be

$$I(t) = \sum_{i=1}^{N} y_i(t), \tag{35}$$

where N is the total number of channels, and y is the single channel current from (1). Only the equilibrium state is considered, so prob [state i at t] $= p_i(\infty)$. If the channels are mutually independent the autocovariance function for the total current will be, for $\tau \geqslant 0$,

$$\begin{aligned} C^2(\tau) &= E\{I(t)\, I(t+\tau) - (E[I(t)])^2\} \\ &= NE\{y(t)\, y(t+\tau) - (E[y(t)])^2\} \\ &= N(V - V_{eq})^2 E\{\gamma(t)\,\gamma(t+\tau) - (E[\gamma(t)])^2\}, \end{aligned} \tag{36}$$

where E denotes statistical expectation. The expectation of any discrete variable, z, is $\Sigma z \,\text{prob}\,(z)$ where the sum is over all possible values of z. With the numbering

convention adopted above, states $1, \ldots, r$ are open states with conductance γ_i ($i = 1, \ldots, r$), and the remaining states are shut ($\gamma_i = 0$). The expectation of the first term in (36) can therefore be written (taking $z = \gamma_i \gamma_j$)

$$N(V - V_{eq})^2 \sum_{i=1}^{r} \sum_{j=1}^{r} \gamma_i \gamma_j \cdot \text{prob}[\text{state } j \text{ at } t+\tau \textit{ and } \text{state } i \text{ at } t]$$
$$= N(V - V_{eq})^2 \Sigma\Sigma \gamma_i \gamma_j \, \text{prob}[\text{state } i \text{ at } t] \cdot \text{prob}[\text{state } j \text{ at } t+\tau | \text{state } i \text{ at } t]$$
$$= N(V - V_{eq})^2 \Sigma\Sigma \gamma_i \gamma_j p_i(\infty) P_{ij}(\tau). \tag{37}$$

The second term in (36) is simply the square of the mean current,

$$N(V - V_{eq}) \Sigma \gamma_i p_i(\infty).$$

These results can be written more compactly in matrix notation using the partitioned matrices defined in (29) and (30). With this notation the autocovariance function becomes

$$C^2(\tau) = N(V - V_{eq})^2 [\boldsymbol{p}_R(\infty) \boldsymbol{\Gamma}_R \boldsymbol{P}_{RR}(\tau) \boldsymbol{\Gamma}_R \boldsymbol{u}_R - (\boldsymbol{p}_R(\infty) \boldsymbol{\Gamma}_R \boldsymbol{u}_R)^2], \tag{38}$$

where $\boldsymbol{u}_R$ is an $r \times 1$ vector with unit elements. Substitution of (18) gives

$$C^2(\tau) = N(V - V_{eq})^2 \left[\boldsymbol{p}_R(\infty) \boldsymbol{\Gamma}_R \left(\sum_{i=1}^{k} \boldsymbol{A}_i e^{\lambda_i \tau} \right)_{RR} \boldsymbol{\Gamma}_R \boldsymbol{u}_R - (\boldsymbol{p}_R(\infty) \boldsymbol{\Gamma}_R \boldsymbol{u}_R)^2 \right]$$
$$= N(V - V_{eq})^2 \boldsymbol{p}_R(\infty) \boldsymbol{\Gamma}_R \left(\sum_{i=2}^{k} \boldsymbol{A}_i e^{\lambda_i \tau} \right)_{RR} \boldsymbol{\Gamma}_R \boldsymbol{u}_R, \tag{39}$$

where the subscript RR denotes the upper left hand $r \times r$ elements, as above.

The second form follows because, from (19),

$$(\boldsymbol{p}_R(\infty) \boldsymbol{\Gamma}_R \boldsymbol{u}_R)^2 = \boldsymbol{p}_R(\infty) \boldsymbol{\Gamma}_R \boldsymbol{A}_1 \boldsymbol{\Gamma}_R \boldsymbol{u}_R.$$

Thus the autocovariance function, like the current relaxation, is the sum of $k-1$ exponentials, with the same rate constants (λ_i) as for current relaxation (equation 28). However the relative contributions of each component to the total will be different, in general, for relaxation and fluctuation measurements.

The autocovariance function may also be written explicitly as the sum of exponential terms (cf. (32), (33) for relaxation)

$$C^2(\tau) = N(V - V_{eq})^2 \sum_{m=2}^{k} b_m e^{\lambda_m \tau}, \tag{40}$$

where the coefficients are now given by

$$b_m = \sum_{i=1}^{r} \sum_{j=1}^{r} p_i(\infty) \gamma_i \gamma_j a_{ij}^{(m)}. \tag{41}$$

If there is only one sort of open state ($r = 1$) with conductance γ we have

$$C^2(\tau) = \gamma(V - V_{eq}) m_I \sum_{i=2}^{k} a_{11}^{(i)} e^{\lambda_i \tau}, \tag{42}$$

where $m_I = Np_R(\infty)(V-V_{eq})\gamma$ is the mean current that is observed to flow at equilibrium.

When there are only $k = 2$ states we need only $a_{11}^{(2)} = p_2(\infty)$ from (20), so

$$C^2(\tau) = \gamma(V-V_{eq})\, m_I p_2(\infty)\, e^{\lambda_2 \tau}. \tag{43}$$

In the simple classical case, $p_2(\infty) = p_T(\infty)$ and $\lambda_2 = -k_2(c+1)$.

Spectral density of current fluctuations

The spectral density function is the Fourier transform of the autocovariance function (see, for example, Bendat & Piersol 1971). The Fourier transform of $e^{|\lambda t|}$ is $-2\lambda/(\lambda^2+\omega^2)$, where $\omega = 2\pi f$, f = frequency. The spectral density function is, therefore, the transform of (39). When multiplied by 2 to give single sided spectral density this is

$$G(f) = 4N(V-V_{eq})^2 \boldsymbol{p}_R(\infty)\boldsymbol{\Gamma}_R\left[\sum_{i=2}^{k} \boldsymbol{A}_i \frac{(-\lambda_i^{-1})}{1+(f/f_i^*)^2}\right]_{RR}\boldsymbol{\Gamma}_R \boldsymbol{u}_R, \tag{44}$$

where $f_i^* = (-\lambda_i)/2\pi$. The spectrum is seen to consist, in general, of the sum of $k-1$ Lorentzian ('$1/f^2$') components with corner frequencies f_i^*.

The spectral density may alternatively be written explicitly as the sum of Lorentzian components

$$G(f) = 4N(V-V_{eq})^2 \sum_{m=2}^{k} b_m \left[\frac{-\lambda_m^{-1}}{1+(2\pi f/\lambda_m)^2}\right], \tag{45}$$

where the coefficients, b_m, are the same as those given for the autocovariance function in (41).

Two special cases of the general result, equation (44), are of particular interest:

(i) If all open states have the same conductance, γ, then (44) becomes

$$G(f) = 4N(V-V_{eq})^2\gamma^2 \boldsymbol{p}_R(\infty)\left[\sum_{i=2}^{k} \boldsymbol{A}_i \frac{(-\lambda_i^{-1})}{1+(f/f_i^*)^2}\right]_{RR}\boldsymbol{u}_R. \tag{46}$$

(ii) If, in addition, there is only one sort of open state ($r = 1$),

$$G(f) = 4\gamma(V-V_{eq})\, m_I\left[\sum_{i=2}^{k} a_{11}^{(i)} \frac{(-\lambda_i^{-1})}{1+(f/f_i^*)^2}\right], \tag{47}$$

where $m_I = Np_R(\infty)(V-V_{eq})\gamma$ is the mean current that is observed to flow at equilibrium.

This result can be applied to the simple classical model, for which, from (20), $a_{11}^{(2)} = (c+1)^{-1} = p_T(\infty)$, the fraction of channels shut at equilibrium, and $\lambda_2 = -k_2(c+1) = -k_2/p_T(\infty)$. Thus, in this case,

$$G(f) = 4\gamma(V-V_{eq})\, m_I \frac{p_T(\infty)/(-\lambda_2)}{1+(f/f^*)^2}. \tag{48}$$

The observed half-power frequency $f^* = -\lambda_2/2\pi$ can thus provide an estimate of λ_2 and thus of $-1/\lambda_2 = k_2^{-1}p_T(\infty)$, where k_2^{-1} is the mean lifetime of the open state.

Alternative forms for the spectral density function

The results for $G(f)$ derived below do not require evaluation of eigenvalues, and may be useful for algebraic treatment of simple cases, or when there are repeated eigenvalues. If the Laplace transform of $\boldsymbol{P}(t)$ is denoted $\boldsymbol{P}^*(s)$, then it follows from (9), since the transform of $\mathrm{d}\boldsymbol{P}/\mathrm{d}t$ is $s\boldsymbol{P}^*(s) - \boldsymbol{P}(0)$, and $\boldsymbol{P}(0) = \boldsymbol{I}$, that

$$\boldsymbol{P}^*(s) = (s\boldsymbol{I} - \boldsymbol{Q})^{-1}. \tag{49}$$

Thus the Fourier transform of (38), in terms of $\omega = 2\pi f$, gives the one sided spectral density as

$$G(\omega) = 2N(V - V_{\mathrm{eq}})^2 \boldsymbol{p}_{\mathrm{R}}(\infty)\boldsymbol{\Gamma}_{\mathrm{R}}\{[(\mathrm{i}\omega\boldsymbol{I} - \boldsymbol{Q})^{-1} + (-\mathrm{i}\omega\boldsymbol{I} - \boldsymbol{Q})^{-1}]_{\mathrm{RR}}\}\boldsymbol{\Gamma}_{\mathrm{R}}\boldsymbol{u}_{\mathrm{R}}, \tag{50}$$

where $\mathrm{i}^2 = -1$ and it is assumed that the d.c. component of the current has been subtracted as is usual. This can also be written as

$$G(\omega) = 4N(V - V_{\mathrm{eq}})^2 \boldsymbol{p}_{\mathrm{R}}(\infty)\boldsymbol{\Gamma}_{\mathrm{R}}\{[-\boldsymbol{Q}(\omega^2\boldsymbol{I} + \boldsymbol{Q}^2)^{-1}]_{\mathrm{RR}}\}\boldsymbol{\Gamma}_{\mathrm{R}}\boldsymbol{u}_{\mathrm{R}}. \tag{51}$$

Another alternative is to use a theorem on partitioned matrices to write

$$\begin{aligned}[(\mathrm{i}\omega - \boldsymbol{Q})^{-1}]_{\mathrm{RR}} &= [\mathrm{i}\omega\boldsymbol{I} - \boldsymbol{Q}_{\mathrm{RR}} - \boldsymbol{Q}_{\mathrm{RT}}(\mathrm{i}\omega\boldsymbol{I} - \boldsymbol{Q}_{\mathrm{TT}})^{-1}\boldsymbol{Q}_{\mathrm{TR}}]^{-1} \\ &= \boldsymbol{Z}(\omega), \quad \text{say.}\end{aligned} \tag{52}$$

Then, from (50),

$$G(\omega) = 2N(V - V_{\mathrm{eq}})^2 \boldsymbol{p}_{\mathrm{R}}(\infty)\boldsymbol{\Gamma}_{\mathrm{R}}[\boldsymbol{Z}(\omega) + \boldsymbol{Z}(-\omega)]\boldsymbol{\Gamma}_{\mathrm{R}}\boldsymbol{u}_{\mathrm{R}}. \tag{53}$$

The variance of current fluctuations, and the single channel conductance

The variance of current fluctuations can be found by direct calculation of the variance from digitized records, or as the area under the spectral density curve. The variance is found as $\int_0^\infty G(f)\,\mathrm{d}f$, from (44) or, equivalently, and more simply, as $C^2(0)$ from (39). Both give, quite generally,

$$\begin{aligned}\mathrm{var}\,[I(t)] &= N(V - V_{\mathrm{eq}})^2 \boldsymbol{p}_{\mathrm{R}}(\infty)\boldsymbol{\Gamma}_{\mathrm{R}}\left[\sum_{i=2}^{k}\boldsymbol{A}_i\right]_{\mathrm{RR}}\boldsymbol{\Gamma}_{\mathrm{R}}\boldsymbol{u}_{\mathrm{R}} \\ &= N(V - V_{\mathrm{eq}})^2[\boldsymbol{p}_{\mathrm{R}}(\infty)\boldsymbol{\Gamma}_{\mathrm{R}}^2\boldsymbol{u}_{\mathrm{R}} - (\boldsymbol{p}_{\mathrm{R}}(\infty)\boldsymbol{\Gamma}_{\mathrm{R}}\boldsymbol{u}_{\mathrm{R}})^2].\end{aligned} \tag{54}$$

However many open states, of whatever lifetime, there may be, if all these open states had the same conductance, γ, equation (54) becomes (since the total fraction of open channels $p_{\mathrm{R}}(\infty) = \boldsymbol{p}_{\mathrm{R}}(\infty)\boldsymbol{u}_{\mathrm{R}}$)

$$\begin{aligned}\mathrm{var}\,[I(t)] &= N(V - V_{\mathrm{eq}})^2\gamma^2 p_{\mathrm{R}}(\infty)[1 - p_{\mathrm{R}}(\infty)] \\ &= \gamma(V - V_{\mathrm{eq}})\,m_I p_{\mathrm{T}}(\infty),\end{aligned} \tag{55}$$

a result which can be obtained directly from the binomial distribution in the case of a single sort of open channel (Stevens 1972). If all open channels have the same conductance, this can therefore be estimated experimentally by the well-known equation

$$\gamma = \frac{\mathrm{var}\,[I(t)]}{m_I(V - V_{\mathrm{eq}})}, \tag{56}$$

provided only that a small fraction of channels is open so that $p_T(\infty) \approx 1$ (see, for example, Anderson & Stevens 1973; Colquhoun *et al.* 1975). Errors in the estimation of channel conductance could occur if (*a*) the recording system did not respond to sufficiently high frequencies, so the variance was underestimated because of loss of high-frequency fluctuations, or (*b*) if any of the open states $(1, \ldots, r)$ were not in fact open all the time, but oscillated rapidly between open and shut states; in this case the conductances inferred from (54), (55) or (56) would be the true open channel conductances multiplied by the fraction of time for which the state is in the open form (an explicit example is given on page 247).

The mean lifetime of the open state

In order to find the distribution of the lifetime of the open conformation, consider a new process in which transition *from* shut states is impossible, i.e. shut states are *absorbing*. (See, for example, Cox & Miller 1965, p. 196.) In this case

$$\boldsymbol{Q}_{TR} = \boldsymbol{Q}_{TT} = \boldsymbol{0}. \tag{57}$$

If the actual process starts in the ith open state, the probability that its lifetime before shutting is equal to or less than t, $F_i(t)$, is then simply the probability, for the new process, that the system is in the shut state at time t. This probability is prob[any shut state at t|open state i at 0], i.e. the ith row of $\boldsymbol{P}_{RT}(t)\,\boldsymbol{u}_T$, where $\boldsymbol{u}_T$ is a column vector with $(k-r)$ unit elements. Thus, if $\boldsymbol{F}(t)$ is the vector of the distribution functions above,

$$\boldsymbol{F}(t) = \boldsymbol{P}_{RT}(t)\,\boldsymbol{u}_T. \tag{58}$$

The corresponding probability density functions, $f_i(t)$, are therefore

$$\boldsymbol{f}(t) = \frac{\mathrm{d}\boldsymbol{P}_{RT}(t)}{\mathrm{d}t}\,\boldsymbol{u}_T \tag{59}$$

$$= \boldsymbol{P}_{RR}(t)\,\boldsymbol{Q}_{RT}\boldsymbol{u}_T, \tag{60}$$

the last relation following, for the new process, from (9) and (57).

Again using (9) and (57), we have, for the new process,

$$\frac{\mathrm{d}\boldsymbol{P}_{RR}(t)}{\mathrm{d}t} = \boldsymbol{P}_{RR}(t)\,\boldsymbol{Q}_{RR}. \tag{61}$$

So, by analogy with (18) the above result can be written

$$\boldsymbol{f}(t) = \left(\sum_{i=1}^{r} \boldsymbol{A}_i' \mathrm{e}^{\lambda_i' t}\right) \boldsymbol{Q}_{RT}\boldsymbol{u}_T, \tag{62}$$

where λ_i' are the eigenvalues, r in number (assumed distinct), of $\boldsymbol{Q}_{RR}$, and $\boldsymbol{A}_i'$ are the corresponding matrices, formed as in (13)–(17), in the expansion $\boldsymbol{Q}_{RR} = \sum_{i=1}^{r} \boldsymbol{A}_i'\lambda_i'$. The mean open lifetimes, m_i, given that the system starts in open state i, could then be found as $m_i = \int_0^\infty t f_i(t)\,\mathrm{d}t$. However, it is more convenient to proceed as follows.

If the Laplace transform of $f(t)$ is denoted $f^*(s)$, then (60) can be written, using (61) with $\boldsymbol{P}(0) = \boldsymbol{I}$,

$$\begin{aligned} \boldsymbol{f}^*(s) &= \boldsymbol{P}^*_{\mathrm{RR}}(s)\, \boldsymbol{Q}_{\mathrm{RT}} \boldsymbol{u}_{\mathrm{T}} \\ &= [s\boldsymbol{I} - \boldsymbol{Q}_{\mathrm{RR}}]^{-1}\, \boldsymbol{Q}_{\mathrm{RT}} \boldsymbol{u}_{\mathrm{T}}. \end{aligned} \tag{63}$$

The inverse transform of this result gives the required distributions, $\boldsymbol{f}(t)$. The mean open lifetimes, m_i, given that the system starts in open state i, are therefore (see, for example, Cox 1962)

$$\boldsymbol{m} = -\left(\frac{\mathrm{d}\boldsymbol{f}^*(s)}{\mathrm{d}s}\right)_{s=0} = \boldsymbol{Q}_{\mathrm{RR}}^{-2}\, \boldsymbol{Q}_{\mathrm{RT}} \boldsymbol{u}_{\mathrm{T}}. \tag{64}$$

To find the distribution of the open lifetime, a weighted combination of the distributions $f_i(t)$ is formed, the weights, π_i, being proportional to the probabilities (in the original process) that, when the channel leaves a shut state, it will enter the ith open state. Thus the row vector $(1 \times r)$ of weights is

$$\boldsymbol{\pi} = \boldsymbol{p}_{\mathrm{T}}(\infty)\, \boldsymbol{Q}_{\mathrm{TR}} / \boldsymbol{p}_{\mathrm{T}}(\infty)\, \boldsymbol{Q}_{\mathrm{TR}} \boldsymbol{u}_{\mathrm{R}}, \tag{65}$$

where $\boldsymbol{p}_{\mathrm{T}}(\infty)$ is the row vector giving the equilibrium fractions of channels in each closed state, and the constant in the denominator is the sum (over open states) of the terms in the numerator so that the sum of the weights is unity.

The probability density function for the lifetime of the open conformation is therefore

$$f_{\mathrm{R}}(t) = \boldsymbol{\pi} \boldsymbol{f}(t), \tag{66}$$

with mean

$$m_{\mathrm{R}} = \boldsymbol{\pi} \boldsymbol{m}. \tag{67}$$

This general result can be simplified, and its physical significance made clearer, if we define a diagonal matrix $\boldsymbol{H}$ with diagonal elements, $h_1, h_2, \ldots, h_r$, that are the row sums of $\boldsymbol{Q}_{\mathrm{RT}}$ and all other elements zero. Thus

$$\boldsymbol{Q}_{\mathrm{RT}} \boldsymbol{u}_{\mathrm{T}} = \boldsymbol{H} \boldsymbol{u}_{\mathrm{R}}, \tag{68}$$

and, because the row sums of $\boldsymbol{Q}$ are zero,

$$(\boldsymbol{Q}_{\mathrm{RR}} + \boldsymbol{H})\, \boldsymbol{u}_{\mathrm{R}} = \boldsymbol{0}, \tag{69}$$

so

$$\boldsymbol{Q}_{\mathrm{RR}}^{-1} \boldsymbol{H} \boldsymbol{u}_{\mathrm{R}} = -\boldsymbol{u}_{\mathrm{R}}. \tag{70}$$

Furthermore, at equilibrium we have, from the line after (22), $\boldsymbol{p}(\infty)\, \boldsymbol{Q} = \boldsymbol{0}$, so

$$\boldsymbol{p}_{\mathrm{R}}(\infty) = -\boldsymbol{p}_{\mathrm{T}}(\infty)\, \boldsymbol{Q}_{\mathrm{TR}} \boldsymbol{Q}_{\mathrm{RR}}^{-1}, \tag{71}$$

and, from (68) and (71), the denominator in (65) can be written as

$$\boldsymbol{p}_{\mathrm{T}}(\infty)\, \boldsymbol{Q}_{\mathrm{TR}} \boldsymbol{u}_{\mathrm{R}} = \boldsymbol{p}_{\mathrm{R}}(\infty)\, \boldsymbol{H} \boldsymbol{u}_{\mathrm{R}}. \tag{72}$$

Combination of (64), (65), (67), (68) and (70)–(72) gives the mean lifetime of the open state as

$$m_{\mathrm{R}} = \frac{\boldsymbol{p}_{\mathrm{R}}(\infty)}{\boldsymbol{p}_{\mathrm{R}}(\infty)\, \boldsymbol{H} \boldsymbol{u}_{\mathrm{R}}}, \tag{73}$$

where $p_R(\infty) = \boldsymbol{p}_R(\infty)\, \boldsymbol{u}_R$ is the total fraction of open channels at equilibrium. The physical significance of this is easy to see if we look at its reciprocal

$$m_R^{-1} = \left(\frac{p_1(\infty)}{p_R(\infty)}\right) h_1 + \left(\frac{p_2(\infty)}{p_R(\infty)}\right) h_2 + \ldots + \left(\frac{p_r(\infty)}{p_R(\infty)}\right) h_r. \tag{74}$$

The quantities h_i are the sums of the rate constants for each path by which the ith open state can shut, and the weighted mean of these quantities is formed, the weights for each being the fraction of the open channels that is in the ith open state at equilibrium.

However many closed states there are, if there is only one open state, (state 1), $r = 1$, so $\boldsymbol{Q}_{RR} = q_{11}$, $\boldsymbol{Q}_{RT}\boldsymbol{u}_T = -q_{11}$ (because the row sums are zero), and $\boldsymbol{\pi} = \pi_1 = 1$. So, from (62) or (63), and (66), the open lifetime has the distribution

$$f_R(t) = f_1(t) = -q_{11}\, e^{q_{11}t}, \tag{75}$$

i.e. the lifetime is exponentially distributed with mean: from (64) and (67),

$$m_R = m_1 = q_{11}^{-2} \cdot (-q_{11}) = -q_{11}^{-1}. \tag{76}$$

In the simple classical case, the open lifetime is thus exponentially distributed with mean $-q_{11}^{-1} = 1/k_2$, as is well known.

In fact it is true quite generally that the lifetime of the ith state is exponentially distributed with mean $-q_{ii}^{-1}$.

The special case of single component spectra

In this case there are only $k = 2$ distinguishable states and the following results hold quite generally:

(i) $n_{12} = p_2(\infty) = q_{11}/(q_{11}+q_{22})$ (see (19)). (77)

(ii) $\lambda_1 = 0, \quad \lambda_2 = q_{11}+q_{22} = q_{11}/p_2(\infty)$ (from (77)). (78)

(iii) $a_{11}^{(2)} = m_{12}n_{21} = q_{11}/(q_{11}+q_{22}) = p_2(\infty)$ from (15) and (20) or (77). (79)

(iv) Mean lifetime of state 1 $= -q_{11}^{-1} = -1/\lambda_2 p_2(\infty)$ from (76) and (78). (80)

(v) The relaxation of the fraction of channels in state 1 is given by (26):

$$p_1(t) = p_1(\infty) + [p_1(0) - p_1(\infty)]\, e^{\lambda_2 t}.$$

(vi) The autocovariance function is

$$C^2(\tau) = \gamma(V - V_{eq})\, m_I\, p_2(\infty)\, e^{\lambda_2 \tau} \tag{81}$$

from (42), (78) and (79).

(vii) The spectral density is, from (47), (77) and (79),

$$G(f) = 4\gamma(V - V_{eq})\, m_I \frac{p_2(\infty)/(-\lambda_2)}{1 + (f/f^*)^2}, \tag{82}$$

where $f^* = -\lambda_2/2\pi$, so mean lifetime of state 1 $= 1/(2\pi f^* p_2(\infty))$, from (80). The conductance γ is the mean conductance of state 1. It would be less than the true single channel conductance if state 1 included shut forms of the channel (see p. 247).

(viii) If state 1 consists of open channels only, and state 2 of shut channels only, so $p_1(\infty) = p_R(\infty)$ and $p_2(\infty) = p_T(\infty)$, then equations (81) and (82) become exactly the results found in the simple classical case, but they are now seen to apply generally to any case with only two distinguishable states (1 = open, 2 = shut) however many *rapidly* interchanging open and shut forms exist. In this case also, the relaxation of membrane current will be given by (34). If there are rapidly interchanging open states with different conductances, γ will be a mean conductance that depends on how much of each sort of open state is present, i.e. it is likely to be concentration dependent (see p. 254).

Similarly, the mean lifetime of the open state can always be found as $1/2\pi f^*$, from (80), as long as a small fraction only of channels is open so $p_T(\infty) \approx 1$, however many rapidly interchanging open and shut forms exist.

This means that a putative low-efficacy drug that could open only a few channels ($p_R \ll 1$), even at high concentrations, must give the correct mean open channel lifetime as $1/2\pi f^*$ whatever detailed theory is postulated (with the assumptions above), and whatever concentration of drug is used. Similarly the assumption that $p_T(\infty) \approx 1$, needed for calculation of the conductance γ from (56), must always be correct for such drugs.

Particular theories of drug action

Some of the recent theories of drug action will now be explored in the light of the results obtained above. All that need be done, in each case, is to write down the Q matrix from the probabilities of transitions between states, then the general results above can be applied. Numerical examples are given to illustrate some properties of the theories, but it is obviously not possible to show all the possibilities. It is not feasible, at this stage, to attempt to fit experimental results; all that can be done is to illustrate some of the less obvious predictions of simple theories.

The KM theory

The simplest way to allow for varying agonist efficacy (though not for co-operativity) is the following scheme, which has been used by a number of authors, e.g. Castillo & Katz (1957), Katz & Miledi (1972), and Anderson & Stevens (1973):

$$\underset{\text{state:}\quad 3}{A + T \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AT} \quad \underset{2}{\overset{\beta}{\underset{\alpha}{\rightleftharpoons}}} \quad \underset{1}{AR}. \tag{83}$$

where, as above, A is the drug (concentration, x_A), T the closed channel and R the open channel. This has $k = 3$ states and therefore, in general, there will be $k - 1 = 2$ components. The solution will be given in full in this case, but in general, when there are more than two components, it will be practicable to use only numerical solutions. The usual deterministic treatment gives the equilibrium occupancies as

$$p_1(\infty) = c/K_c d; \quad p_2(\infty) = c/d; \quad p_3(\infty) = 1/d; \tag{84}$$

where $d = [c(K_c+1)/K_c]+1$, (85)

$K_c = \alpha/\beta$ (equilibrium constant for conformation change),

$c = x_A/K_T$ (normalized concentration),

$K_T = k_2/k_1$ (equilibrium constant for binding to shut channel).

From (11),

$$\boldsymbol{Q} = \begin{bmatrix} -\alpha & \alpha & 0 \\ \beta & -(\beta+k_2) & k_2 \\ 0 & k_2 c & -k_2 c \end{bmatrix}. \tag{86}$$

As expected, $\det(\boldsymbol{Q}) = 0$, and the eigenvalues are $\lambda_1 = 0$,

$$\lambda_2, \lambda_3 = \tfrac{1}{2}[-b \pm (b^2 - 4\alpha k_2 d)^{\frac{1}{2}}], \tag{87}$$

where $b = \beta(K_c+1)+k_2(c+1)$.

It may be noted that

$$\lambda_2+\lambda_3 = -b; \quad \lambda_2\lambda_3 = \alpha k_2 d. \tag{88}$$

There is only one open state, so, from (75) and (76), its lifetime is exponentially distributed with mean α^{-1}, i.e. the mean open lifetime is not concentration dependent, which it is in some more complicated theories.

The matrix $\boldsymbol{M}$, the columns of which are the eigenvectors of $\boldsymbol{Q}$, is

$$\boldsymbol{M} = \begin{bmatrix} 1 & 1 & 1 \\ 1 & (1+\lambda_2/\alpha) & (1+\lambda_3/\alpha) \\ 1 & \left(\dfrac{1+\lambda_2/\alpha}{1+\lambda_2/k_2 c}\right) & \left(\dfrac{1+\lambda_3/\alpha}{1+\lambda_3/k_2 c}\right) \end{bmatrix}. \tag{89}$$

The determinant of $\boldsymbol{M}$, after some manipulation by the use of (88), becomes

$$D = (\lambda_3-\lambda_2)\,d/\alpha. \tag{90}$$

The inverse of $\boldsymbol{M}$, which can clearly be found accurately only if λ_2 and λ_3 differ sufficiently, is thus

$$\boldsymbol{N} = \boldsymbol{M}^{-1} = \begin{bmatrix} p_1(\infty) & p_2(\infty) & p_3(\infty) \\ \dfrac{\lambda_3/\alpha+1}{D(k_2c/\lambda_3+1)} & \dfrac{k_2c/\alpha-1}{D(k_2c/\lambda_3+1)} & \dfrac{-\lambda_3}{D\alpha} \\ \dfrac{-(\lambda_2/\alpha+1)}{D(k_2c/\lambda_2+1)} & \dfrac{-(k_2c/\alpha-1)}{D(k_2c/\lambda_2+1)} & \dfrac{\lambda_2}{D\alpha} \end{bmatrix}. \tag{91}$$

From this and $\boldsymbol{M}$, the $\boldsymbol{A}_i$ needed to complete the result for relaxation of the membrane current (28) can be found with the use of (15). There is only one sort of open state, so (47) can be used for the spectral density of current fluctuations. It gives

$$G(f) = 4\gamma(V-V_{eq})\,m_I\left[a_{11}^{(2)}\frac{(-\lambda_2)^{-1}}{1+(2\pi f/\lambda_2)^2}+a_{11}^{(3)}\frac{(-\lambda_3)^{-1}}{1+(2\pi f/\lambda_3)^2}\right], \tag{92}$$

where, from (15) and (87)–(91) (denoting elements of $\boldsymbol{M}$ and $\boldsymbol{N}$ as m_{ij} and n_{ij}),

$$a_{11}^{(2)} = m_{12}n_{21} = n_{21} = \frac{(\lambda_3/\alpha + 1)}{D(k_2 c/\lambda_3 + 1)}; \tag{93}$$

$$a_{11}^{(3)} = m_{13}n_{31} = n_{31} = \frac{-(\lambda_2/\alpha + 1)}{D(k_2 c/\lambda_2 + 1)}. \tag{94}$$

The case of fast binding

If the binding step is sufficiently rapid, in this case if $k_2(c+1) \gg (\alpha+\beta)$, as is commonly assumed (see, for example, Anderson & Stevens 1973), then states 2 and 3 will cease to be 'kinetically distinguishable', so they can be pooled into a single state, and the states relabelled thus:

$$\underset{\text{state:}}{\underbrace{\text{A} + \text{T} \overset{\text{fast}}{\rightleftharpoons} \text{AT}}_{2}} \overset{\beta}{\underset{\alpha}{\rightleftharpoons}} \underset{1}{\text{AR}}. \tag{95}$$

The general procedure for obtaining the transition probabilities (and hence the $\boldsymbol{Q}$ matrix) when compound states such as state 2 (above) are involved, can be illustrated by this example. The probability of a $1 \to 2$ transition is proportional to α, exactly as before, but the probability of a $2 \to 1$ transition must be found as follows. If we define event A as 'state 1 at time $t + \Delta t$', event B as 'state 2 at time t', event B_1 as 'state 2 is the AT form at time t' (i.e. in the form which can go directly to state 1) and event B_2 as 'state 2 is not in the AT form at time t' then, because B_1 and B_2 are mutually exclusive, the rules of probability give the required probability as

$$P[A|B] = (P[A|B_1] \cdot P[B_1] + P[A|B_2] \cdot P[B_2])/P[B] \tag{96}$$

$$= P[A|B_1]\,(P[B_1]/P[B]) \tag{97}$$

$$= \beta \times (\text{equilibrium fraction of state 2, i.e. of shut channels, that are in the AT form}). \tag{98}$$

The second line follows (when $\Delta t \to 0$) because no direct transition is possible between T and AR so $P[A|B_2] \to 0$. This gives the intuitively obvious result in the last line. In the present case this is, from (84),

$$\beta[(c/d)/(c/d + 1/d)] = \beta c/(c+1).$$

Thus we find, when binding is sufficiently fast,

$$\boldsymbol{Q} = \begin{bmatrix} -\alpha & \alpha \\ \dfrac{\beta c}{c+1} & -\dfrac{\beta c}{c+1} \end{bmatrix}. \tag{99}$$

In this case there are only two kinetically distinguishable states, $k = 2$; state 1 is open, and state 2 consists only of shut states. The single component relaxation and

noise spectra are found simply by substitution into the results in paragraphs (i)–(viii) on pp. 243–244. Substituting from (99) into (78) and (80) shows, for example, that the mean lifetime of the open state is $1/\alpha$, and the rate constant in the relaxation and noise spectra is $\lambda_2 = -[\alpha + \beta c/(c+1)] = -\alpha/p_T(\infty)$.

The case of fast conformation change

If, contrary to the case in the last section, the slow step were drug *binding*, and the conformation change were relatively fast, i.e. $(\alpha+\beta) \gg k_2(c+1)$, then there would again be only two kinetically distinguishable states, and correspondingly a single component spectral density function. The states must now be labelled thus:

$$\begin{array}{lcl} & \overset{k_1}{\underset{k_2}{\rightleftharpoons}} & \overset{\text{fast}}{\rightleftharpoons} \\ \text{A} + \text{T} & \underbrace{\qquad\qquad} & \underbrace{\text{AR}} \\ \text{state:} \quad 2 & & 1 \end{array} \tag{100}$$

State 1 is now the occupied state rather than the open state: it is open for a fraction $\beta/(\alpha+\beta)$ of the time only. This complicates the interpretation somewhat, because it is current rather than occupancy that can be observed. An analysis exactly analogous with that just given for the fast binding case gives

$$\boldsymbol{Q} = \begin{bmatrix} -k_2\alpha/(\alpha+\beta) & k_2\alpha/(\alpha+\beta) \\ k_2 c & -k_2 c \end{bmatrix}. \tag{101}$$

Thus, from (78) the observed rate constant $\lambda_2 = -[k_2\alpha/(\alpha+\beta)]/p_2(\infty)$, which can be estimated from the autocovariance function (81), or spectral density function (82), will provide an estimate of the mean lifetime of state 1, the *occupied* state, if $p_2(\infty) \approx 1$, i.e. if most receptors are *vacant*. The mean conductance of state 1, γ, can be estimated from (56) if most receptors are vacant, but it would have to be divided by $\beta/(\alpha+\beta)$, which cannot be estimated from fluctuation data, to obtain the true conductance of the open channel.

Some numerical examples

In this, and other numerical examples, we shall take realistic values for the mean lifetime of the open state $m_R = 1$ ms, $N = 10^7$, $(V - V_{eq}) = -80$ mV, and $\gamma = 25$ pS; but no attempt will be made here to fit experimental results. We shall consider full agonists as capable of opening 95 % of channels at sufficiently high concentrations and hypothetical partial agonists that can open only 20 % or 5 % of channels.

(1) *Full agonist, low concentration.* For $m_R = 1$ ms we need $\alpha = 1000\,\text{s}^{-1}$. To open 95 % of channels it is necessary to take $K_c = 5.263 \times 10^{-2}$, so $\beta = 1.9 \times 10^4\,\text{s}^{-1}$. A moderately low concentration, $c = 2.6 \times 10^{-3}$, will open, at equilibrium, 4.7 % of channels, $p_R(\infty) = p_1(\infty) = 0.047$ (i.e. about 5 % of those that can be opened). This results in $m_I = 940$ nA, a large current by experimental standards. If we are interested in fast binding, it might seem reasonable to take the dissociation rate constant for binding as $k_2 = 10^4\,\text{s}^{-1}$ (cf. $\alpha = 10^3\,\text{s}^{-1}$). The predictions for these values are shown in figure 1. Both fluctuations and relaxation show hardly any

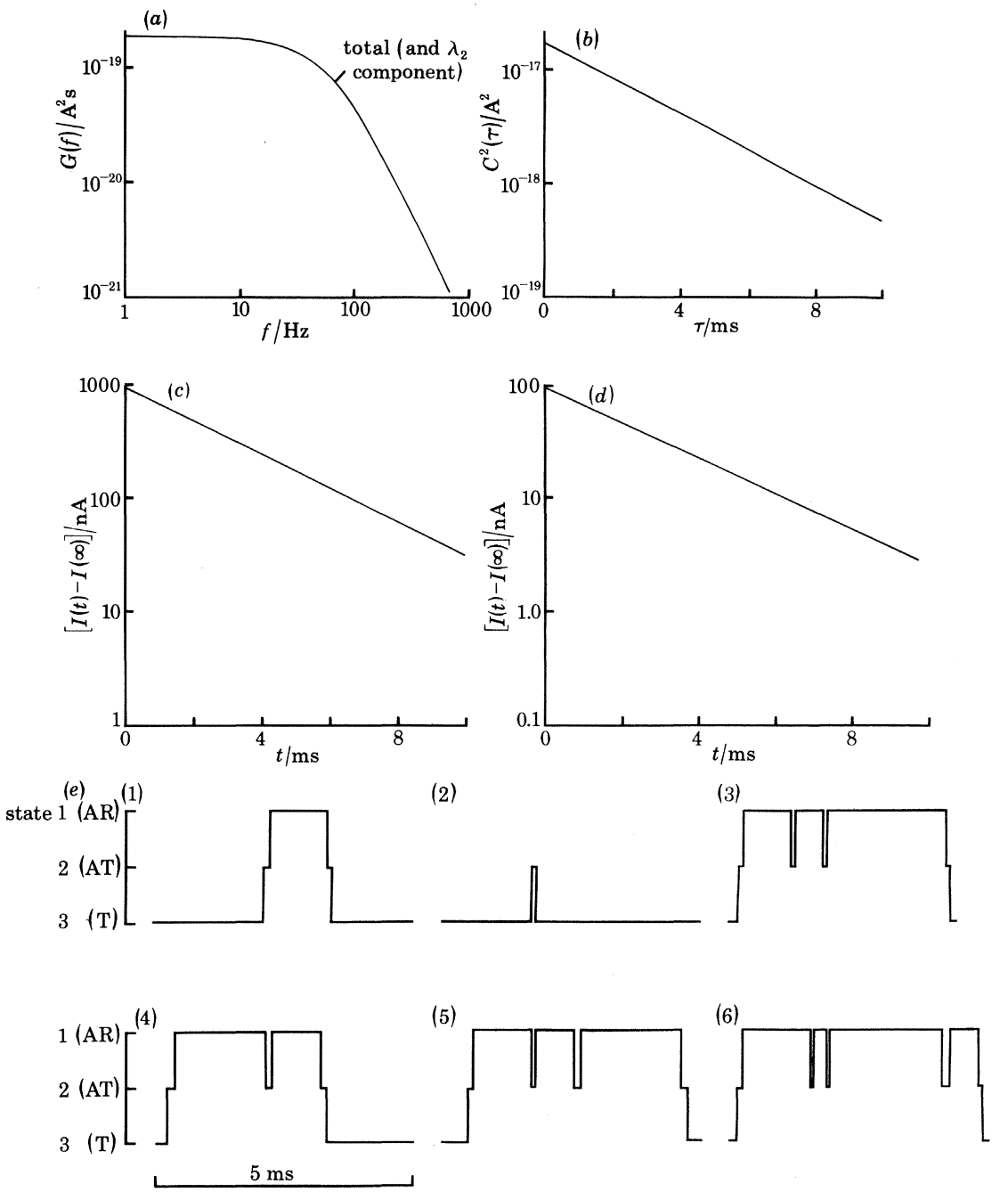

FIGURE 1. For description see opposite.

deviation from a single component with rate constant $-\lambda_2 = 354.5\,s^{-1}$ for fluctuations (figure 1*a*, *b*), and $-\lambda_2 = 337.1\,s^{-1}$ for 'offset relaxation' (figure 1*c*) when the concentration is suddenly reduced to zero (which may mimic the decay phase of a miniature endplate current). With lower concentrations both rate constants would approach the latter value. In each case $-\lambda_3$ was very large, and could not be observed. It is seen that, despite the single component and fairly rapid binding, the observed rate constant overestimates the mean lifetime by a factor of about 3, and the offset relaxation time constant is slightly less than that for fluctuations. In fact k_2 would have to be much larger, 10^5 or $10^6\,s^{-1}$ rather than $10^4\,s^{-1}$, to approach the true fast binding limit, i.e. to make $k_2(c+1) \gg (\alpha+\beta)$, but there is an upper limit to the rate that can be postulated. For example, if $k_2 = 10^6\,s^{-1}$ then, if the drug concentration were 10 μM, this would imply $k_1 = 2.6 \times 10^8\,M^{-1}\,s^{-1}$ which approaches the upper limit for association rate set by diffusion, and is higher than is actually observed for small molecule–macromolecule association (see, for example, Burgen 1966).

Figure 1*d* shows the relaxation of membrane current that would follow a step change from $\alpha = 900\,s^{-1}$ to $\alpha = 1000\,s^{-1}$ (simulation of a voltage jump). This implies that p_1 changes from $p_1(0) = 0.0519$ to $p_1(\infty) = 0.047$. The rate constants are, of course, the same as in figure 1*a* and *b*. Again the slow component only is visible.

The physical interpretation of this example can be made much clearer with the aid of the examples of simulated channel behaviour shown in figure 1*e*. When a molecule is bound, it will rapidly dissociate again (mean lifetime $k_2^{-1} = 0.1$ ms) *if* the channel does not happen to open, as in figure 1*e* (2). However, in this example, the AT state is more likely to open than to dissociate, by a factor β/k_2 ($= 1.9$ in this case). It may open only once during a given occupancy, as in figure 1*e* (1), or it may open several times, as in figure 1*e* (3)–(6). The probability, π say, that the next transition of a channel in state 2 (AT) will be opening (to state 1, AR) is, in this theory, $\pi = \beta/(k_2+\beta)$, and the probability that its next transition is dissociation (to state 3) will be $1-\pi$. Therefore the probability of observing r openings during a given occupancy is $P(r) = \pi^r(1-\pi)$, a geometric distribution ($r = 0, 1, 2, \ldots, \infty$). The mean

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FIGURE 1. The KM theory. Full agonist ($K_c = 5.26 \times 10^{-2}$, $\alpha = 1000\,s^{-1}$, $\beta = 1.9 \times 10^4\,s^{-1}$, $c = 2.6 \times 10^{-3}$, $k_2 = 10^4\,s^{-1}$, $p_1(\infty) = 0.047$, $p_2(\infty) = 0.002$, $p_3(\infty) = 0.951$. (*a*) Spectral density against frequency; double logarithmic plot; $\lambda_2 = -354.5\,s^{-1}$, $\lambda_3 = -29671.4\,s^{-1}$. (*b*) Autocovariance function, $C^2(\tau)$, against τ; semilogarithmic plot; λ_2 and λ_3 as in (*a*). (*c*) Current relaxation after sudden reduction of agonist concentration to zero at $t = 0$ ('offset'); semilogarithmic plot; $\lambda_2 = -337.1\,s^{-1}$, $\lambda_3 = -29662.9\,s^{-1}$. (*d*) Current relaxation after voltage jump simulated by a step change in α from $900\,s^{-1}$ to $1000\,s^{-1}$ at $t = 0$; semilogarithmic plot; $p_1(0) = 0.0519$, $p_2(0) = 0.002$, $p_3(0) = 0.946$; λ_2 and λ_3 as in (*a*). (*e*) Simulation (with the aid of a random number generator) of the behaviour of a single channel. Six examples are shown of the events following the binding of a drug molecule. The number (r) of channel openings before dissociation, in these examples are: $r = 0$ in (2); $r = 1$ in (1), $r = 2$ in (4); $r = 3$ in (3) and (5); $r = 4$ in (6). The mean number of openings is 1.9, and the mean number given that at least one opening occurs is 2.9 (see text).

number of openings per occupancy, $\Sigma rP(r)$, is therefore $\pi/(1-\pi) = \beta/k_2$, i.e. 1.9 openings per occupancy in this example, the mean length of each opening being 1 ms. This may be compared with the mean duration of the occupied (AT or AR) state which is 2.00 ms in this case (from an analogue of (74), or from (80) and (101)). This

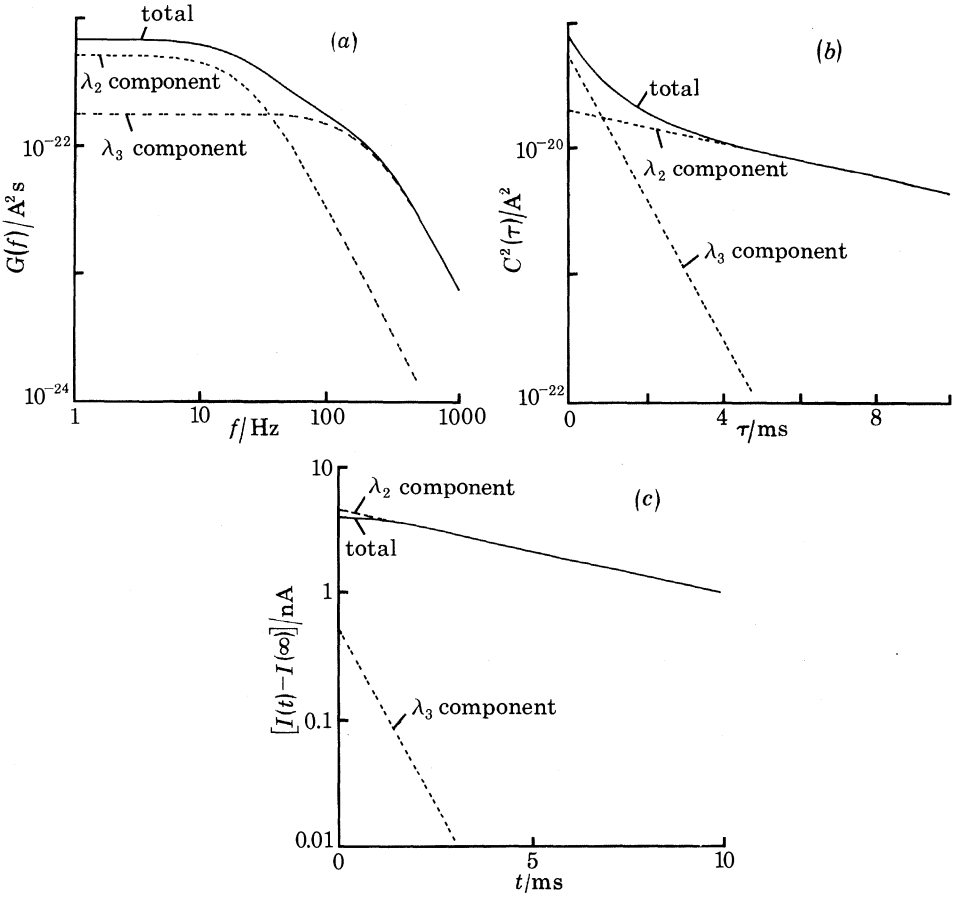


FIGURE 2. The KM theory. Moderate agonist ($K_c = 4, \alpha = 1000\ s^{-1}, \beta = 250\ s^{-1}, c = 8 \times 10^4$, $k_2 = 200\ s^{-1}$; $p_1(\infty) = 2 \times 10^{-4}$, $p_2(\infty) = 8 \times 10^{-4}$, $p_3(\infty) = 0.999$. (*a*) Spectral density against frequency; double logarithmic plot; $\lambda_2 = -154.5\ s^{-1}$, $\lambda_3 = -1295.6\ s^{-1}$, (*b*) Autocovariance function, $C^2(\tau)$ against τ; semilogarithmic plot; λ_2 and λ_3 as in (*a*). (*c*) Current relaxation after sudden reduction of agonist concentration to zero at $t = 0$ ('offset'); semilogarithmic plot; $\lambda_2 = -154.4\ s^{-1}$, $\lambda_3 = -1295.6\ s^{-1}$.

example is for a strong agonist, and it is seen from figure 1*e* that the channel is indeed open for most of the time that it is occupied. Occupancies without opening, as in figure 1*e* (2), make no contribution to the observed current. The current occurs mainly in rapid bursts of openings that occur during a single occupancy; the probability of r openings per occupancy, *given* that at least one opening takes place, is $\pi^{r-1}(1-\pi)$, with mean $1/(1-\pi)$. For the KM theory this is $(1+\beta/k_2)$, which is 2.9

openings, of mean duration 1 ms each, in this case. The apparent 'open lifetime' of $\lambda_2^{-1} = 2.82$ ms (from figures 1*a*, *b* or *c*) is therefore hardly surprising.

(2) *Moderate agonist, low concentration, slow binding.* Take $K_c = 4$ so that, at high concentrations, 20% of channels can be opened, and $c = 8 \times 10^{-4}$ so that at equilibrium $p_R(\infty) = 2 \times 10^{-4}$. This gives $m_I = 4$ nA, similar to the peak current caused by release of a single quantum of acetylcholine. As before $\alpha = 1000\,s^{-1}$, but now suppose drug dissociation is relatively slow, $k_2 = 200\,s^{-1}$. The predictions are shown in figure 2. Two components can be seen clearly in spectral density (figure 2*a*) and autocorrelation (figure 2*b*) functions, with

$$-\lambda_2 = 154.5\,s^{-1} \quad \text{and} \quad -\lambda_3 = 1295.6\,s^{-1}.$$

Neither of these estimates the mean open lifetime accurately, though λ_2 is not far from the mean *occupied* lifetime (see equation (80)). In contrast the slow component, $-\lambda_2 = 154.4\,s^{-1}$, is predominant in the offset relaxation curve (figure 2*c*). The fast component is only visible at short times and would be difficult to observe.

(3) *Weak agonist, low concentration, slow binding.* Take $K_c = 19$ so that at high concentrations only 5% of channels can be opened, and $c = 0{\cdot}05$ so $p_1(\infty) = p_R(\infty) = 0.0025$ (5% of openable channels), $p_2(\infty) = 0.0475$, $p_3(\infty) = 0.95$. As before $\alpha = 1000\,s^{-1}$ and drug dissociation is relatively slow, $k_2 = 250\,s^{-1}$. The calculations, shown in figure 3, demonstrate that the spectral density (figure 3*a*) shows predominantly the high-frequency component, $-\lambda_3 = 1068.9\,s^{-1}$, as does the autocorrelation function (figure 3*b*) over the first decade at least; the slow component, $-\lambda_2 = 246.2\,s^{-1}$, is smaller. So a tolerable estimate of mean open lifetime would be found from fluctuation measurements in this case. In the offset relaxation curve (figure 3*c*) the rate constants are similar, $-\lambda_2 = 233.9\,s^{-1}$ and $-\lambda_3 = 1068.7\,s^{-1}$, but it is the *slow* ($\lambda_2$) component that predominates except at short times. The slow component, in this case, reflects mainly the lifetime of the occupied state.

The physical interpretation of this example can be clarified by the methods that were used for example 1. In this case there is a high ratio ($= K_c = 19$) of AT (state 2) to AR (state 1) at equilibrium. The majority of drug bindings are followed by dissociation with no channel opening; the probability of $r = 0$ openings is

$$P(0) = (1 - \pi) = k_2/(k_2 + \beta) = 0.83.$$

If there is no opening the mean occupied lifetime will be $k_2^{-1} = 4.0$ ms. When a channel does open (for a mean open lifetime of 1 ms) there will rarely be more than one opening per occupancy (and, when there is, the openings will often not be in quick succession), so it is not surprising that fluctuation measurements give a tolerable estimate of the mean open lifetime. On the other hand the offset relaxation curve is slow. It reflects largely the mean lifetime of the occupied state which is 4.21 ms, and the reason for this is apparent from the simulation in figure 3*d*. Any channel not occupied at $t = 0$, the moment of drug removal, can never become occupied. Figure 3*d* shows four examples of the evolution of events following $t = 0$ for channels that *were* occupied at $t = 0$. In figures 3*d* (1) and 3*d* (4), the drug dissociates with no opening; in figure 3*d* (2) the channel was open at $t = 0$ but it soon

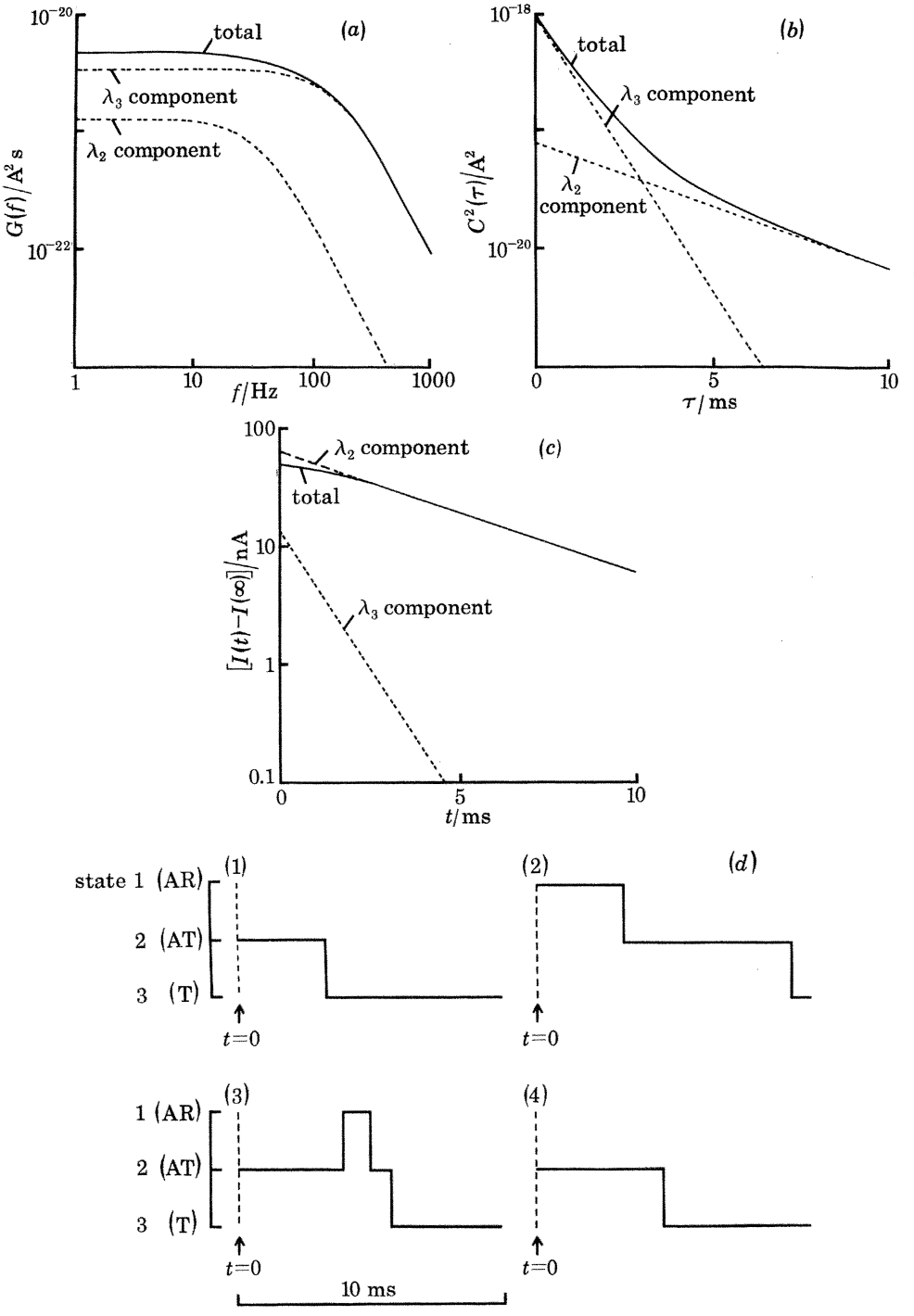


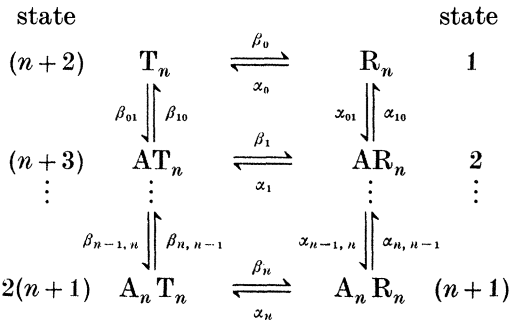
FIGURE 3. For description see opposite.

closes, so this channel would contribute to the early part of the relaxation current. However, in figure 3*d* (3) the channel remains shut (AT) for several milliseconds after $t = 0$, before opening; channels like this will result in the relaxation current being prolonged, and reflecting the mean occupied lifetime (4.21 ms), which may be compared with the time constant for the predominant component in figure 3*c*, which is $-\lambda_2^{-1} = 4.28$ ms.

The Monod–Wyman–Changeux theory

This theory (Monod, Wyman & Changeux 1965), which has been widely applied to enzymes and haemoglobin, has been considered as one of the simplest theories that can account, by a physically plausible mechanism, for the observations that drugs can open ion channels, that the response shows cooperativity, and that drugs vary in efficacy (Karlin 1967; Podleski & Changeux 1970; Colquhoun 1973, 1975; Thron 1973; Rang 1975). The observed degree of cooperativity would suggest *at least* two subunits, but even when only two subunits are considered, the number of rate constants involved is too large for the theory to be applied to the acetylcholine receptor at the moment. Nevertheless the general theory will be considered first, and then a simplification of it, which is simple enough for plausible rate constants to be assigned for numerical calculations.

If n subunits are considered, the theory can be summarized thus:



(102)

The matrix $\boldsymbol{Q}$ is most easily given in its partitioned form:

$$\boldsymbol{Q}_{\mathrm{RT}} = \mathrm{diag}\,(\alpha_0, \alpha_1, \ldots, \alpha_n); \tag{103}$$

$$\boldsymbol{Q}_{\mathrm{TR}} = \mathrm{diag}\,(\beta_0, \beta_1, \ldots, \beta_n). \tag{104}$$

FIGURE 3. The KM theory. Weak agonist ($K_c = 19$, $\alpha = 1000\ \mathrm{s}^{-1}$, $\beta = 52.63\ \mathrm{s}^{-1}$, $c = 5 \times 10^{-2}$, $k_2 = 250\ \mathrm{s}^{-1}$); $p_1(\infty) = 0.0025$, $p_2(\infty) = 0.0475$, $p_3(\infty) = 0{\cdot}95$. (*a*) Spectral density against frequency; double logarithmic plot; $\lambda_2 = -246.2\ \mathrm{s}^{-1}$, $\lambda_3 = -1068.9\ \mathrm{s}^{-1}$. (*b*) Autocovariance function, $C^2(\tau)$ against τ; semilogarithmic plot; λ_2 and λ_3 as in (*a*). (*c*) Current relaxation after sudden reduction of agonist concentration to zero at $t = 0$ ('offset'); semilogarithmic plot; $\lambda_2 = -233.9\ \mathrm{s}^{-1}$, $\lambda_3 = -1068.7\ \mathrm{s}^{-1}$. (*d*) Simulation (with the aid of a random number generator) of the behaviour of a single channel when the drug concentration is suddenly reduced to zero at $t = 0$, so binding of drug becomes impossible after $t = 0$. Four examples are shown of the events following drug removal for cases where drug was bound at $t = 0$.

Q_{RR} is tridiagonal, the main diagonal elements being $-(\alpha_i + i\alpha_{i,i-1} + (n-i)\alpha_{i,i+1}x_A)$ for $0 \leqslant i \leqslant n$; the elements of the diagonal above this are $(n-i)\alpha_{i,i+1}x_A$ for $0 \leqslant i \leqslant n-1$, and those of the diagonal below it are $i\alpha_{i,i-1}$ for $1 \leqslant i \leqslant n$, where x_A is the drug concentration. All other elements are zero. Q_{TT} is the same as Q_{RR} but with β in place of α. There are thus, in general, $k = 2(n+1)$ states so the relaxation function and spectral density function will have $2n+1$ components. The deterministic treatment (see, for example, Monod *et al.* 1965; Karlin 1967) shows that not all rate constants are independent; for example the microscopic equilibrium constants for binding are $K_R = \alpha_{i,i-1}/\alpha_{i-1,i}$, $K_T = \beta_{i,i-1}/\beta_{i-1,i}$ for $1 \leqslant i \leqslant n$, and $\alpha_i/\beta_i = LM^i$ for $1 \leqslant i \leqslant n$, where $M = K_R/K_T$ and $L = \alpha_0/\beta_0$. Nevertheless, the number of rate constants involved is too large for this theory to be used at the moment.

The mean lifetime of the open state can be inferred from (73), and is

$$m_R = \frac{(1+c_R)^n}{\sum_{i=0}^{n} \binom{n}{i} c_R^i \alpha_i}, \tag{105}$$

where $c_R = x_A/K_R$ is the drug concentration normalized with respect to the equilibrium constant (K_R) for binding to the open conformation. As would be expected this changes progressively from $1/\alpha_0$ at zero drug concentration to $1/\alpha_n$ at sufficiently high drug concentration. If all α_i are the same, α say, it would reduce simply to $1/\alpha$, independent of concentration.

In practice it is most common to observe fluctuation spectra that deviate only slightly from a single component. If, for example, all the binding steps were fast there would be, effectively, only $k = 2$ states, open (R_n, AR_n, ..., $A_n R_n$, state 1) and shut (T_n, AT_n, ..., $A_n T_n$, state 2). By an extension of (96), and the solution for the fractions of each species at equilibrium, we obtain

$$-q_{11} = q_{12} = \sum_{0}^{n} \binom{n}{i} c_R^i \alpha_i/(1+c_R)^n,$$

and

$$-q_{22} = q_{21} = \sum_{0}^{n} \binom{n}{i} c_T^i \beta_i/(1+c_T)^n,$$

where $c_T = x_A/K_T$ is the drug concentration normalized with respect to the equilibrium constant (K_T) for binding to the shut conformation. As expected from (80), $-q_{11}^{-1}$ is the mean lifetime of the open state already derived in (105). The rate constant for the predicted single component spectra (equations (34), (81) and (82)), is $\lambda_2 = q_{11} + q_{22} = q_{11}/p_T(\infty)$ from (78). This value has also been given, in a slightly different context, by Hammes & Wu (1974). Thus, as before, the correct mean lifetime (105) would be inferred from fluctuation measurements, provided only that a small fraction of channels were open at equilibrium. If the various open forms had different conductances, the mean conductance would be dependent on the fraction of each open form in state 1, for example, dependent on drug concentration and membrane potential.

A simplified Monod–Wyman–Changeux theory

Although, from thermodynamic considerations, open channels must exist in the absence of drug, the fraction of such channels is small, at the muscle endplate at least. If they are neglected, and only two subunits are considered, we have

$$\begin{array}{ccc} & \mathrm{T_2} & \\ & \beta_{01}\downarrow\uparrow\beta_{10} & \\ & \beta_1 & \\ \mathrm{AT_2} & \rightleftharpoons & \mathrm{AR_2} \\ & \alpha_1 & \\ \beta_{12}\downarrow\uparrow\beta_{21} & & \alpha_{12}\downarrow\uparrow\alpha_{21} \\ & \beta_2 & \\ \mathrm{A_2T_2} & \rightleftharpoons & \mathrm{A_2R_2} \\ & \alpha_2 & \end{array} \tag{106}$$

This has five states in general. Further simplification can be achieved by assuming that binding to the shut (T) conformation is very rapid, though binding to the open (R) conformation, for which the drug affinity is greater, may not be. This gives $k = 3$ states: state 1, A_2R_2 (open); state 2, AR_2 (open); and state 3, T_2, AT_2 and A_2T_2 (shut). There will thus generally be two components in the spectra and relaxation curves in this case. Deterministic solution at equilibrium gives

$$p_1(\infty) = c_T^2/K_2 d; \quad p_2(\infty) = 2c_T/K_1 d; \quad p_3(\infty) = (c_T+1)^2/d, \tag{107}$$

where $d = (c_T+1)^2 + 2c_T/K_1 + c_T^2/K_2$ and $K_i = \alpha_i/\beta_i$ so $K_2 = MK_1$. Other symbols are defined as above. Thus, using (97) for transitions from the composite state 3, we have

$$\boldsymbol{Q} = \begin{bmatrix} -(2\alpha_{21}+\alpha_2) & 2\alpha_{21} & \alpha_2 \\ \alpha_{21}c_T/M & -(\alpha_1+\alpha_{21}c_T/M) & \alpha_1 \\ \beta_2\left(\dfrac{c_T}{1+c_T}\right)^2 & \dfrac{\beta_1 2c_T}{(1+c_T)^2} & -\left(\dfrac{\beta_2 c_T^2 + 2\beta_1 c_T}{(1+c_T)^2}\right) \end{bmatrix}. \tag{108}$$

The mean open lifetime, from equation (73), is

$$m_R = \frac{2Mc_T + c_T^2}{\alpha_1 2Mc_T + \alpha_2 c_T^2}, \tag{109}$$

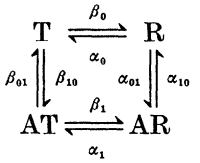
which changes from α_1^{-1} to α_2^{-1} as concentration increases, as expected. This mean lifetime would reduce to α^{-1} if $\alpha_2 = \alpha_1$ ($= \alpha$ say), i.e. if the shutting rates were the same for both sorts of occupied channel (one or two agonist molecules), only their opening rates differing. Less obviously if, as well as $\alpha_1 = \alpha_2$, it were also true that $\gamma_1 = \gamma_2$ (both open states have the same conductance), then the form of $\boldsymbol{Q}$ (108) would be such that the coefficients of the λ_3 component (b_3, (33) and (41)) vanish. Therefore the fluctuation and relaxation spectra would appear to have a single (λ_2) component; and furthermore $\lambda_2 = -\alpha/p_T(\infty)$ in this case, as in the case of genuine one component spectra (80), so the correct mean lifetime could be estimated as usual, *regardless* of how slow the binding to the open channel may be.

If binding were very rapid to *both* open and shut conformations there would be only two distinguishable states, with $q_{12} = -q_{11} = (\alpha_1 2Mc_T + \alpha_2 c_T^2)/(2Mc_T + c_T^2)$,

and $q_{21} = -q_{22} = (\beta_1 2c_T + \beta_2 c_T^2)/(1+c_T)^2$. The single rate constant would, from (78), be $\lambda_2 = (q_{11}+q_{22})$, and the mean open lifetime $m_R = -q_{11}^{-1}$, from (76) which agrees with the result in (109). Furthermore, from (80), $m_R = -1/\lambda_2 p_T(\infty)$, which, if $\alpha_1 = \alpha_2 = \alpha$ would become simply α^{-1} since in this case $\lambda_2 = -\alpha/p_T(\infty)$.

Theories that postulate independent subunits

Another simple explanation of many experimental observations may be obtained by postulating subunits that, far from undergoing a concerted conformation change as in the Monod–Wyman–Changeux theory, are quite independent of each other (see, for example, Colquhoun, 1973; Adams, 1975*b*,*c*). For example one might postulate n independent subunits, each being like a single Monod–Wyman–Changeux unit thus:



(110)

Or subunits like a single KM unit (83) which is the same except that open, unoccupied channels are neglected. It is further postulated, by analogy with the Hodgkin–Huxley (1952) theory of axonal ion channels, that all n subunits must be in the R conformation (R or AR) for a channel to open. With n identical and equivalent subunits, each of which may be R, AR, T or AT, there will be $n+1$ distinguishable open states (i.e. all n subunits in the R conformation; $0, 1, \ldots, n$ subunits occupied by agonist molecules), and a considerable number of shut states (e.g. 7 for $n = 2$, 15 for $n = 3$). This is more states than in the case of the Monod–Wyman–Changeux theory. If the Q matrix is written down it can be found from (73) that the mean lifetime of the open state is, in general,

$$m_R = \frac{(1+c_R)}{n(\alpha_0 + c_R \alpha_1)}, \tag{111}$$

which changes progressively from $1/n\alpha_0$ at zero concentration to $1/n\alpha_1$ at sufficiently high drug concentration.

If we consider two independent KM subunits (83) there will clearly be only one open state, (AR, AR) and

$$Q = \begin{array}{c} \begin{matrix} (AR,AR) & (AR,AT) & (AR,T) & (AT,AT) & (AT,T) & (T,T) \end{matrix} \\ \left[\begin{array}{c|cccccc} -2\alpha & 2\alpha & 0 & 0 & 0 & 0 \\ \hline \beta & -(\beta+k_2+\alpha) & k_2 & \alpha & 0 & 0 \\ 0 & k_1 x_A & -(k_1 x_A + \alpha) & 0 & \alpha & 0 \\ 0 & 2\beta & 0 & -2(\beta+k_2) & 2k_2 & 0 \\ 0 & 0 & \beta & k_1 x_A & -(\beta + k_1 x_A + k_2) & k_2 \\ 0 & 0 & 0 & 0 & 2k_1 x_A & -2k_1 x_A \end{array}\right] \end{array} \tag{112}$$

Thus, from (73) or (76), the mean lifetime of the open state is simply $1/2\alpha$ (or, for n such subunits, $1/n\alpha$), which is not concentration-dependent. There are however six states and thus five eigenvalues (λ_i) so, in general, the fluctuation spectra and relaxation curve would have five components; and their rate constants (λ_i) would not be related simply to the mean open lifetime.

Two state subunits: the fast binding case

The theory can be further simplified by postulating that each (independent and equivalent) subunit has only two states, e.g. that all binding stages are fast so that for each subunit only the R and the T conformation are kinetically distinguishable states. The system is now directly analogous with that postulated for the Hodgkin–Huxley potassium channel, and results like those given by Chen & Hill (1973) and by Conti & Wanke (1975) are obtained ((115)–(118) below). With n subunits there will be $k = n+1$ states altogether, namely, R^n (state 1, open), ..., $R^{n-i}T^i$, ..., T^n (state k). All elements of $\boldsymbol{Q}$ will be zero except for the diagonal elements (chosen to make the row sums zero), the supradiagonal $q_{i,i+1} = (n-i+1)y_{12}$, and the subdiagonal $q_{i+1,i} = iy_{21}$ where $i = 1, ..., n$ and y_{12} and y_{21} are the rate constants for R→T and T→R, respectively, in a single subunit. For example, for Monod–Wyman–Changeux subunits (110) with fast binding, $y_{12} = (\alpha_0+\alpha_1 c_R)/(1+c_R)$ and $y_{21} = (\beta_0+\beta_1 c_T)/(1+c_T)$, from (96), and from the fraction of each species at equilibrium. In the simpler case of KM subunits (83) with fast binding we have (see (99)) $y_{12} = \alpha$, $y_{21} = \beta c_T/(c_T+1)$. The mean open lifetime, from (76), is $-q_{11}^{-1} = 1/ny_{12}$, which gives the same results as already found above for these two cases. As an example, if there are $n = 2$ subunits,

$$\boldsymbol{Q} = \begin{array}{c} \begin{matrix} \quad(\mathrm{R},\mathrm{R}) & \quad(\mathrm{R},\mathrm{T}) & \quad(\mathrm{T},\mathrm{T}) \end{matrix} \\ \left[\begin{array}{c:cc} -2y_{12} & 2y_{12} & 0 \\ \hdashline y_{21} & -(y_{21}+y_{12}) & y_{12} \\ 0 & 2y_{21} & -2y_{21} \end{array}\right] \end{array}. \tag{113}$$

Although even this simplest case has more states than its analogue in the Monod–Wyman–Changeux theory, there are a number of simplifying relations in the present case. It can be shown that the n eigenvalues of $\boldsymbol{Q}$ are simply related thus:

$$\lambda_{i+1} = -i(y_{12}+y_{21}) \quad (i = 1, ..., n), \tag{114}$$

($\lambda_1 = 0$ as usual). So there is, as might be expected, only one estimatable rate constant, $(y_{12}+y_{21})$.

Relaxation. The relaxation rate can be found from the general result (31), but it can be found more simply by multiplying the independent probabilities that a single subunit is in the R conformation, i.e. by raising (34) to the power n, giving

$$\begin{aligned} I(t) &= I(\infty)\,(1+\theta\,\mathrm{e}^{\lambda t})^n \\ &= I(\infty)\left(1+\sum_{i=1}^{n}\binom{n}{i}\theta^i\,\mathrm{e}^{i\lambda t}\right), \end{aligned} \tag{115}$$

where $$\theta = \frac{p_R(0) - p_R(\infty)}{p_R(\infty)} \quad \text{and} \quad \lambda = -(y_{12} + y_{21}). \tag{116}$$

This makes it clear why the eigenvalues are as given in (114). It also shows that the weight attached to the component with rate constant $-i\lambda$ is $\binom{n}{i}\theta^i$. Thus if $\theta \ll 1$, i.e. if the perturbation changes the number of R subunits by only a small amount relative to its eventual value, the component with the slowest rate constant, $-\lambda$, will predominate (see figure 4*d*).

Fluctuations. In this case $\boldsymbol{M}$ (and therefore $\boldsymbol{M}^{-1} = \boldsymbol{N}$) turn out to be symmetrical. Therefore not only does $\sum_{i=2}^{k} a_{11}^{(i)} = 1 - p_{open}(\infty) = p_2(\infty) + p_3(\infty) + \ldots + p_k(\infty)$ as usual (from (20)), but, from the fact that $a_{11}^{(j)} = n_{j1}$ and (because $\boldsymbol{N}$ is symmetrical) $n_{j1} = n_{1j}$, it follows from (19) that

$$a_{11}^{(i)} = p_i(\infty),$$

so the weights attached to each rate constant (114) in the autocovariance function (42) are simply the fractions of shut states, $p_2(\infty), p_3(\infty), \ldots, p_k(\infty)$.

The autocovariance function can therefore be written explicitly, by using these relations, and (42), as

$$\begin{aligned} C^2(\tau) &= \gamma(V - V_{eq}) m_I [p_2(\infty)\, e^{\lambda\tau} + \ldots + p_k(\infty)\, e^{n\lambda\tau}] \\ &= m_I^2 [(1 + \rho\, e^{\lambda\tau})^n - 1]/N, \end{aligned} \tag{117}$$

where $\lambda = -(y_{12} + y_{21})$, $\rho = p_T(\infty)/p_R(\infty) = y_{12}/y_{21}$, and p_T and p_R are the fractions of *individual subunits* in the T and R conformations. The second form follows because $p_{i+1} = \binom{n}{i} p_R^{n-i} p_T^i$ $(i = 0, 1, \ldots, n)$.

Similarly, the spectral density function will be, from the above relations, and (47),

$$\begin{aligned} G(f) &= 4\gamma(V - V_{eq})\, m_I \sum_{i=1}^{n} \binom{n}{i} p_R^{n-i} p_T^i \left[\frac{-(i\lambda)^{-1}}{1 + \left(\frac{2\pi f}{i\lambda}\right)^2}\right] \\ &= \frac{4m_I^2}{N\lambda} \sum_{i=1}^{n} \binom{n}{i} \frac{\rho^i}{i\left[1 + \frac{2\pi f}{i\lambda}\right]^2}. \end{aligned} \tag{118}$$

If the nature and concentration of the drug are such that most of the subunits are in the T conformation (a stronger condition than $p_{open} \ll 1$) then $p_k(\infty) \approx 1$ and $y_{12} \gg y_{21}$. Therefore most weight will be attached to the highest frequency component, and the autocovariance function, and spectral density, will approximate to a single component with rate constant $-n\lambda = n(y_{12} + y_{21}) \approx n y_{12}$ as illustrated in figures 4*a* and *b*. Thus, under these circumstances, fluctuation measurements would give a good estimate of the mean open lifetime of the channel. It would certainly be expected that low drug concentrations should approach this condition and so give a rate constant of $-n\lambda$. However, it has been observed by Neher &

Sakman (1975) that the smallest perturbations tested gave a rate constant for relaxation that was very similar to those observed for fluctuation measurements, whereas for sufficiently small perturbations a rate constant of $-\lambda$, i.e. n times slower, is predicted (see above). This certainly suggests that the simplest version

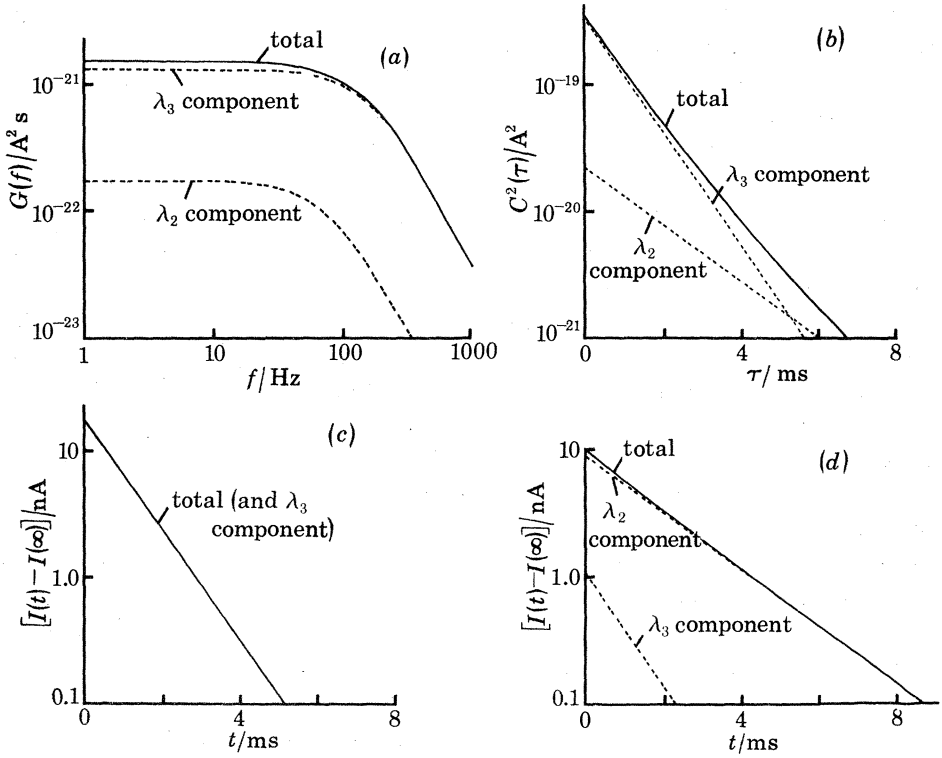


FIGURE 4. Two independent KM subunits (binding assumed very rapid, and channel open only when both subunits have the R conformation). For each subunit $K_c = 2.6 \times 10^{-2}$, $\alpha = 500\ s^{-1}$, $\beta = 1.9 \times 10^{-4}\ s^{-1}$, thus mean channel lifetime $= 10^{-3}$ s; $c_T = 8.3 \times 10^{-4}$ so $p_1(\infty) = 0.95 \times 10^{-3}$, $p_2(\infty) = 0.0597$, $p_3(\infty) = 0.939$. (*a*) Spectral density against frequency; double logarithmic plot; $\lambda_2 = -515.9\ s^{-1}$, $\lambda_3 = -1031.8\ s^{-1}$. (*b*) Autocovariance function, $C^2(\tau)$, against τ; semilogarithmic plot; λ_2 and λ_3 as in (*a*). (*c*) Current relaxation after sudden reduction of agonist concentration to zero at $t = 0$ ('offset'); semilogarithmic plot; $\lambda_2 = -500\ s^{-1}$, $\lambda_3 = -1000\ s^{-1}$. (*d*) Current relaxation after voltage jump simulated by a step change in α from $400\ s^{-1}$ to $500\ s^{-1}$ at $t = 0$; semilogarithmic plot; $p_1(0) = 1.46 \times 10^{-3}$, $p_2(0) = 0.0735$, $p_3(0) = 0.925$; λ_2 and λ_3 as in (*a*).

of the independent subunit theory, discussed above, may be wrong. A similar inference has been drawn by P. R. Adams (personal communication), for similar reasons.

The offset relaxation curve, i.e. that predicted when the agonist concentration is reduced suddenly to zero, is shown in figure 4*c*. In this case the curve (115) reduces to a single component with rate constant $-\lambda = n\alpha$, close to that for fluctuations, but n-fold faster than for relaxation after a small perturbation.

Discussion

It is the main object of this work to provide a general method of calculation of the results expected, on the basis of theories of drug action, in noise and relaxation experiments.

The numerical examples illustrate the complicated behaviour that could potentially result even from quite simple theories, and they underline the obvious fact that such experiments alone cannot distinguish between all rival theories. It would clearly be very helpful if it were possible to measure the concentration dependence, if any, of the rate constants (λ_i) and of the single channel conductance (γ). But, so far, measurements have been technically possible only over a limited concentration range, and concentration dependence has not been measurable in muscle tissue. However, in electroplaques of the electric eel (*Electrophorus*) Sheridan & Lester (1975) have been able to measure the concentration dependence of the relaxation rate, and these measurements promise to yield very interesting results. The examples illustrate the fact that it is quite possible in principle for different rate constants to predominate in fluctuation and relaxation experiments. The fact that this has not yet been observed experimentally can be interpreted as evidence against simple forms, at least, of theories involving independent subunits. It is also clear that apparently single-component spectra can give entirely wrong channel lifetimes and conductances.

On the other hand, if there are only two kinetically distinguishable states, a large class of theories gives results that have a simple interpretation (pp. 243–244) if the fraction of open channels is small (a condition that is necessarily satisfied by a hyphothetical weak partial agonist).

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