INTERPRETING POWER SPECTRA FROM NONSTATIONARY MEMBRANE CURRENT FLUCTUATIONS

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ABSTRACT It is often desirable to characterize membrane current fluctuations from ionic channels under conditions in which the mean current and the variance of the fluctuations change with time. A simple theory is developed that relates the power spectrum to the channel characteristics under such nonstationary conditions, assuming that the mean current time-course has been removed from the fluctuation records. Strategies for removing the mean time-course are discussed, and spectra are calculated from simulated channel fluctuations for comparison with the theory.

INTRODUCTION

Stationary noise analysis has become a standard method for determining kinetic characteristics and the conductance of single ionic channels in biological membranes (see reviews: Neher and Stevens, 1977; DeFelice, 1977; Chen, 1978). In this method the channels are placed in a steady, partly activated state, and spontaneous fluctuations in the steady membrane current are analyzed, usually by taking the power spectrum. A major difficulty in applying this technique has been the maintainance of a steady state: chemically activated channels desensitize and voltage-activated channels inactivate, resulting in drifting base lines that contaminate the power spectra.

One solution to this problem of nonstationarity in the data is to apply an explicitly nonstationary analysis technique to a set of current records elicited by identical stimuli (Sigworth, 1980, 1981). In this technique a mean time-course is subtracted from each of a group of records, and from the fluctuations that remain, a two-dimensional covariance function is computed. Unfortunately, in practice, several hundred records are needed to provide an interpretable covariance function (see Sigworth, 1981), because only ensemble averaging is used in computing the function. In contrast, stationary noise analysis requires only some tens of records (or segments of one long current record) because the one-dimensional covariance function is computed as a time-average over the duration of the record, and needs to be averaged over fewer records for an equivalent amount of scatter in the result.

This paper considers a hybrid of the stationary and nonstationary methods which has already been applied to fluctuations from Na^+ channels (Conti et al., 1980) and K^+ channels[1] in axons. A set of current records is obtained, and the mean current time-course is estimated

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[1] Begenesich, T., C. F. Stevens, and F. Sigworth. Unpublished results.

and is subtracted from each record. The average power spectrum is then computed from the fluctuations that remain after the subtraction. Conti et al. (1980) have shown how this can be done in practice, and have presented the theory for predicting the spectrum from specific models of channel gating. This paper will show that the spectrum from nonstationary fluctuations can be interpreted in the same way as a spectrum from stationary fluctuations, given a special assumption – that there is only one kinetically distinct open state of a channel.

The main experimental requirements for the technique are that the current records must be long enough to avoid errors in the low-frequency points of the spectrum, and that a provision be made for estimating and removing the changes in the mean current that would otherwise contaminate the spectrum. Although in the past the technique was used with currents that showed slow drifts with time, no restriction on the degree of nonstationarity is imposed by the theory, and repeatable currents that show large and rapid changes in time can be analyzed.

An example of an experimental protocol for studying voltage-activated channels would be the following: A series of channel-activating membrane depolarizations is applied for a sufficiently long initial period to allow slow inactivation processes to reach a nearly steady-state. After this initial period the pulses are continued while the responses are recorded. The analysis of these data then starts with the removal of the mean time-course from each record; a simple way to do this is to subtract pairs of records (Conti et al., 1980). This results in residuals that have zero mean but twice the variance of the individual records; the average power spectrum is then calculated from these residuals. To this a correction is added, consisting of the scaled power spectrum of the mean-current time-course. The result is a spectrum that can be readily compared with the predictions of specific gating schemes.

THEORY

The usual goals of fluctuation analysis are to estimate the single-channel current i, the number of channels N, and the time-course of relaxations in the channel-gating process. In this section we will show how the spectrum depends on these properties. We make the usual simplifying assumptions that the channels are identical, function independently, and have only one nonzero conductance level corresponding to the current i at the test potential. The ensemble expectation value $I(t)$ of the membrane current is then proportional to the probability $p(t)$ of a channel being open,

$$I(t) = Nip(t). \tag{1}$$

We also make the special assumption that, of all the kinetically distinguishable states of a channel's gating machinery, only one corresponds to the open channel. Calling this state 1, we define the conditional probability function $p_{11}(t + \tau | t)$ that a channel is open at time $t + \tau$ given that it is open at t. The assumption that the open state is unique, plus the usual assumption (stated explicitly in Colquhoun and Hawkes, 1977) that the transitions among states constitute a Markov process with time-independent rates, imply that $p_{11}(t + \tau | t)$ is independent of t for positive time displacements τ (see, for example, Cox and Miller, 1965). This is equivalent to saying that, given that a channel is in its open state at time t, the probability that after a delay it will also be found in the open state depends only on the length of the delay; it does not depend on t or the pattern of transitions before time t. We can then for convenience define an even function ϕ such that

$$\phi(\tau) = p_{11}(t + \tau \mid t) \qquad \tau \geqslant 0$$

and

$$\phi(\tau) = \phi(-\tau). \tag{2}$$

We will be using this function later to replace p_{11}; we can do this because for negative values of τ we can reconstruct p_{11} by Bayes' rule (Feller, 1968):

$$p_{11}(t + \tau \mid t) = \frac{p(t + \tau)}{p(t)} \phi(\tau) \qquad \tau \leq 0. \tag{3}$$

The function ϕ represents the kinetic information to be obtained from the fluctuation analysis. For stationary fluctuations, because p is constant, Eq. 3 shows that $\phi(\tau)$ is identical to the conditional probability $p_{11}(t + \tau \mid \bar{t})$ for all values of τ. In the stationary case ϕ is also proportional, within a constant, to the covariance $C(\tau)$ of the fluctuations (Stevens, 1972),

$$C(\tau) = Ni^2 p\phi(\tau) - Ni^2 p^2 \tag{4}$$

Covariance of nonstationary fluctuations

In the general nonstationary case a covariance function that depends on two time variables is required (Sigworth, 1981). Let $y(t)$ be an individual current record, and $x(t) = y(t) - I(t)$ be its fluctuation away from the ensemble expectation $I(t)$, as illustrated in Fig. 1 *A*. Then the nonstationary covariance is $C(t_1,t_2) = \langle x(t_1)x(t_2) \rangle$, where brackets indicate the ensemble expectation value. An equation analogous to Eq. 4 relates $C(t_1,t_2)$ to the probability functions (see Sigworth, 1981, for a derivation):

$$C(t + \tau,t) = Ni^2 p(t)\, p_{11}(t + \tau \mid t) - Ni^2 p(t)p(t + \tau). \tag{5}$$

Inasmuch as the stationary covariance $C(\tau)$ is a time-average of the nonstationary covariance,

$$C(\tau) = \lim_{T \to \infty} \frac{1}{T} \int_0^T C(t + \tau,t)\mathrm{d}t,$$

our plan is to form a similar time-average of Eq. 5 to obtain quantities that depend only on τ. To have the result apply to practical situations, we assume that the current records $y(t)$ have a fixed length T. Without loss of generality we can extend the data functions y and x and the underlying probability functions p and p_{11} outside the interval $[0,T]$ by making them periodic with period T to form the functions y_T, x_T, p_T, and p_{11T} (Fig. 1 *B*). This will be useful when we take Fourier tranforms later to compute the power spectrum. The desired time-average can then be taken over one period:

$$\left\langle \frac{1}{T} \int_0^T x_T(t)x_T(t + \tau)\mathrm{d}t \right\rangle = Ni^2 \frac{1}{T} \int_0^T p_T(t)p_{11T}(t + \tau \mid t)\mathrm{d}t - Ni^2 \frac{1}{T} \int_0^T p_T(t)p_T(t + \tau)\mathrm{d}t. \tag{6}$$

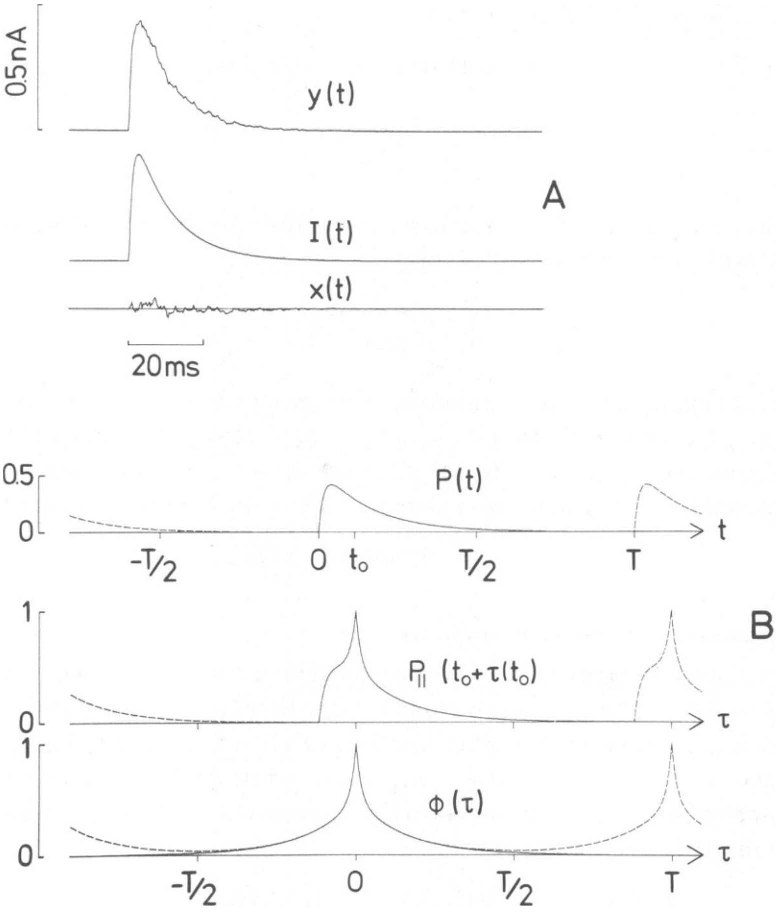

FIGURE 1 Example of the various functions for a particular gating scheme (the one used in Example 2). *A*, an individual current record $y(t)$, its ensemble average $I(t)$ and the difference $x(t)$. *B*, the probability functions p, p_{11} and $\phi(\tau)$ from the same scheme plotted on a different time scale with $T = 64$ ms. The corresponding periodic functions p_t, p_{11T} and $\tilde{\phi}(\tau)$ are superimposed (dashed curves). $\phi(\tau)$ in this scheme is the sum of two exponentials with equal amplitudes and with time constants of 1 and 10 ms.

The integral on the left and the integral in the second term on the right are the finite autocorrelations R_x and R_p of x_T and p_T, so Eq. 6 can be rewritten

$$\langle R_x(\tau) \rangle = Ni^2\bar{p}\tilde{\phi}(\tau) - Ni^2R_p(\tau) \tag{7}$$

where $\bar{p}$ is the time-average of $p(t)$,

$$\bar{p} = \frac{1}{T}\int_0^T p(t)\mathrm{d}t,$$

and the function $\tilde{\phi}$ is defined by

$$\tilde{\phi}(\tau) = (1/\bar{p}T)\int_0^T p_T(t)p_{11T}(t + \tau \mid t)\mathrm{d}t \tag{8}$$

and is useful because it is an approximation to $\phi(\tau)$. Because $p_{\mathrm{II}T}$ is periodic, $\tilde{\phi}$ is also periodic with period T, and is an even function. To show that it approximates $\phi(\tau)$ on the interval $-T/2 \leq \tau \leq T/2$, Eq. 8 can be rewritten using Eqs. 2 and 3, for $0 \leq \tau \leq T$,

$$\tilde{\phi}(\tau) = \frac{1}{\bar{p}T}\int_0^{T-\tau} p(t)p_{\mathrm{I}}(t+\tau\,|\,t)\mathrm{d}t + \frac{1}{\bar{p}T}\int_{T-\tau}^{T} p(t)p_{\mathrm{II}}(t+\tau-T\,|\,t)\mathrm{d}t$$

$$= \phi(\tau)\frac{1}{\bar{p}T}\int_0^{T-\tau} p(t)\mathrm{d}t + \phi(T-\tau)\frac{1}{\bar{p}T}\int_0^{\tau} p(t)\mathrm{d}t. \quad (9)$$

Inspection shows that $\tilde{\phi}$ approaches ϕ if ϕ decays to zero on a time-scale short compared with T. In practice T can usually be chosen so that the difference between $\tilde{\phi}$ and ϕ is negligible. This simplifies the analysis, because the function ϕ from a particular theory of channel gating can then be inserted directly into Eq. 7. As an example, Fig. 1 B shows ϕ and $\tilde{\phi}$ functions corresponding to the simulated data in Fig. 2 B. The record duration $T = 64$ ms was chosen to be relatively short (not much longer than the longest time constant of 10 ms in the ϕ function). With this value of T a small difference between the curves is discernable in the region $-T/2 \leq \tau \leq T/2$.

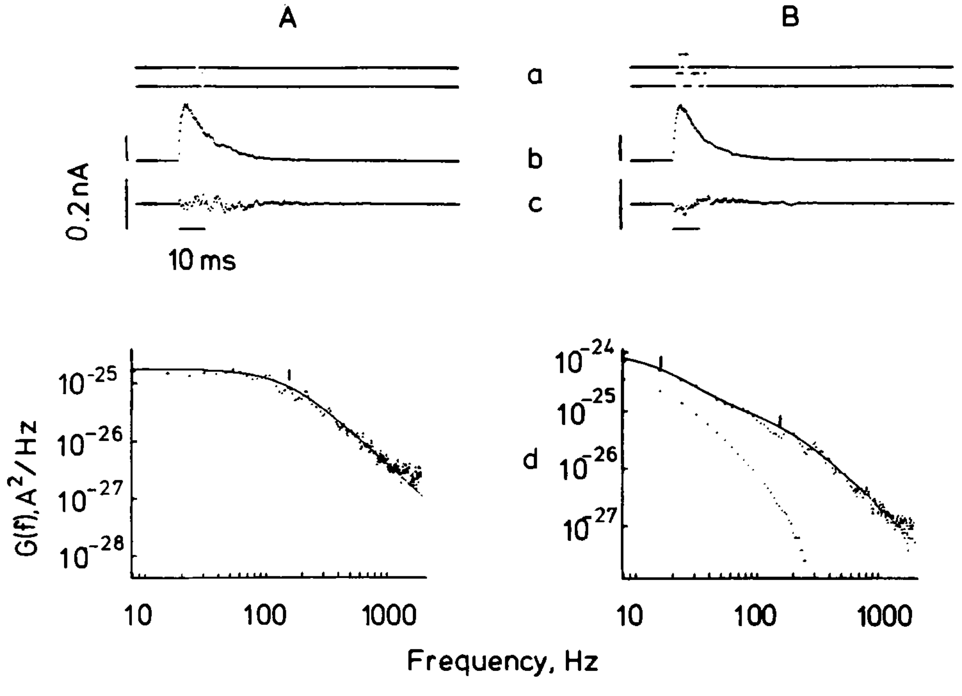

FIGURE 2 Simulated membrane currents and the resulting power spectra from schemes A and B. (a) Representative single-channel currents. (b) A typical "membrane current record" formed by summing the currents in ~ 5,500 channels in scheme A, or exactly 1,000 channels in scheme B. (c) Differences of two current records, shown at a larger vertical scale. (d) Power spectra computed from 50 difference records according to Eq. 17, with the predictions of Eqs. 21 and 23 shown as smooth curves. The dotted curve in B is the correction term $G_l(f)/N$ that was added to $G_x(f)$ before plotting the spectrum. The flattening of the high-frequency roll-off in the spectrum in (A) is probably due to frequency-domain aliasing since no filtering was performed on the simulated currents. The disaveraging procedure preserved fluctuations at zero frequency, and the resulting spectral values at $f = 0$ (not plotted) were close to the theoretical values in each case.

Eq. 7 is the analogue of Eq. 4 that we have been seeking, since the quantity $<R_x>$ on the left-hand side is the covariance of x on the interval $[0,T]$, which can be determined experimentally, and ϕ and P can be computed for particular models of the gating process.

Power Spectra

In actual experiments the average power spectrum, rather than the covariance, is usually calculated. We can obtain an expression for the power spectrum by taking the discrete Fourier transform of Eq. 7. We assume that the data consist of M discrete samples at times $t = 0, \delta_t, 2\delta_t, \dots (M-1)\delta_t$ where $M\ \delta_t = T$. The discrete Fourier transform of a function $w_T(t)$ is defined as (Brigham, 1974)

$$\mathbf{D}\{w_T(t)\} = \sum_{k=0}^{M-1} w(k\delta_t)\exp(-j2\pi fk\delta_t);$$

where f takes the values $0, \delta_f, 2\delta_f, \dots (M-1)\delta_f$, with $\delta_f = 1/T$. Operating on Eq. 7 with the normalized, one-sided transform $(2/M)$ **D** gives the equation for the average power spectrum $<G_x>$ of the fluctuations $x(t)$,

$$\langle G_x(f)\rangle = Ni^2\overline{p}\tilde{\Phi}(f) - Ni^2G_p(f) \qquad (10)$$

where in this and succeeding equations f takes on only the values $0, \delta_f, 2\delta_f, \dots M/2\delta_f$. Each term in Eq. 10 is real-valued, and only half of the frequency components need to be kept because each term of Eq. 7 is real-valued and even. In writing Eq. 10 we have defined the one-sided spectrum G_w of a function $w_T(t)$,

$$G_w(f) = \frac{2}{M^2}|\mathbf{D}\{w_T(t)\}|^2,$$

and have used the property that it is also the transformed autocorrelation,

$$G_w(f) = \frac{2}{M}\mathbf{D}\{R_w(\tau)\}.$$

The function $\tilde{\Phi}$ is the transform of $\tilde{\phi}$

$$\tilde{\Phi} = \frac{2}{M}\mathbf{D}\{\tilde{\phi}(\tau)\}.$$

If $\tilde{\phi}$ is band-limited, then for the discrete values of f, the continuous Fourier transform can be used to evaluate $\tilde{\Phi}$ (Brigham, 1974),

$$\tilde{\Phi}(f) = 2\int_{-T/2}^{T/2} \tilde{\phi}(\tau)\cos(2\pi f\tau)\mathrm{d}\tau \qquad (11)$$

(only the cosine part is needed because $\tilde{\phi}$ is even). $\tilde{\Phi}$ is approximately equal to the continuous Fourier transform of ϕ,

$$\Phi(f) = 2\int_{-\infty}^{\infty} \phi(\tau)\cos(2\pi f\tau)\mathrm{d}\tau \qquad (12)$$

which, for simple theories of channel gating, can be evaluated analytically.

The Correction Term

Eq. 10 shows the relationship between the average power spectrum $\langle G_x \rangle$, which can be determined experimentally, and the Fourier transform $\tilde{\Phi}(f)$ of the decay function $\tilde{\phi}(\tau)$, which can be evaluated for particular gating schemes. This is the basic relationship for interpreting both stationary and nonstationary power spectra. In the stationary case the additional term in Eq. 10, which involves the power spectrum G_p of the probability $p(t)$, is usually ignored. Because p is constant in this case, G_p is zero at all frequencies except $f = 0$. (At $f = 0$, $G_p = 2\bar{p}^2$). The zero-frequency point in a measured power spectrum is usually discarded because of high-pass filtering. Thus the G_p term does not affect the part of the spectrum to be interpreted, which is proportional to $\tilde{\Phi}$.

In the nonstationary case G_p contributes to the measured spectrum at all frequencies. Its contribution is small when p is nearly constant (the nearly-stationary case) or when p is small. When p is not small, G_p can be estimated in a straightforward way from the experimental data since, from Eq. 1

$$G_p(f) = \frac{G_I(f)}{N^2 i^2},$$

where $G_I(f)$ is the power spectrum of the mean current time-course $I(t)$. Defining $\bar{I} = Ni\bar{p}$ as the time-averaged mean current in the interval $[0,T]$, we can rewrite Eq. 10,

$$i\bar{I}\tilde{\Phi}(f) = \langle G_x(f) \rangle + \frac{1}{N} G_I(f). \tag{13}$$

The term $N^{-1}G_I(f)$ is the "correction term" that can be added to the average power spectrum. The result is a "corrected spectrum" which is proportional to $\tilde{\Phi}(f)$, just as in the stationary case. The correction term vanishes in the limit of small p; in Eq. 13 this limit corresponds to letting $N \rightarrow \infty$ while I (and therefore G_I) remain bounded.

The Average Variance

Integrating Eq. 10 over frequency is equivalent to evaluating Eq. 7 at $\tau = 0$, yielding an expression for the time-averaged variance of x, $\overline{\sigma_x^2}$:

$$\overline{\sigma_x^2} = Ni^2\bar{p} - Ni^2\overline{p^2}, \tag{14}$$

where bars indicate time averages over $[0,T]$ as before. The ratio of the variance to the mean current is

$$\frac{\overline{\sigma_x^2}}{\bar{I}} = i\left[1 - \frac{\overline{p^2}}{\bar{p}}\right] \tag{15}$$

and can be used to estimate i.

Removal of the Mean Time-Course

The preceding derivations assumed that we have fluctuation records $x(t)$ obtained by subtracting the noiseless ensemble mean current $I(t)$ from individual records. In practice $I(t)$ is not known exactly, but can be approximated by $I_n(t)$, the average of a group of n

successively evoked current records y_i. If this average is then subtracted from the same individual records, the average power spectrum for that group can be computed as a sum of the spectra of the residual fluctuations. Letting $\mathbf{G} = (2/M^2)|\mathbf{D}|^2$ be the power spectrum operator,

$$\langle G_x(f)\rangle = \left\langle \frac{1}{n-1}\sum_{i=1}^{n} \mathbf{G}\{y_i(t) - I_n(t)\}\right\rangle. \tag{16}$$

Notice that the normalizing factor is $1/(n-1)$ instead of $1/n$ because of the correlation in fluctuations between the y_i and I_n. Subtracting pairs of records is a special case where $n = 2$ and the two terms of the sum are identical so that (Conti et al., 1980)

$$\langle G_x(f)\rangle = \frac{1}{2}\langle \mathbf{G}\{y_{i+1}(\mathrm{t}) - y_i(\mathrm{t})\}\rangle \tag{17}$$

but twice as many records are required to obtain the same variance in G_x as would be required in Eq. 16 with a large value of n. This can be understood from the fact that from $2m$ records only m statistically independent differences can be formed.

In some situations a set of successively evoked records is not available for estimating the mean time-course. An example is a single long record obtained from the bath application of an agonist to channels that desensitize. If the desensitization time-course is slow compared with the fluctuations of interest, the slow trend can be removed by high-pass filtering the signal or by fitting a function to the slowly changing base-line. Both of these techniques alter the low-frequency components of the spectrum; the remainder, however, can be corrected and interpreted according to Eq. 13.

COMPUTER SIMULATIONS

I found the result described by Eq. 13 very surprising, since it implies that $\phi(\tau)$ or $\Phi(f)$ can be estimated from a set of current records with any mean-current time-course $I(t)$, as long as the mean is subtracted from each fluctuation record. As examples, therefore, I have made two simulations of current fluctuations in the extremely nonstationary case where the mean current rises and decays to zero within the analysis interval. The kinetic characteristics of the simulated channels were different in the two simulations, but the rate constants and numbers of channels were chosen to give the same time-course for $I(t)$. As will be seen, different spectra are obtained for the two schemes, reflecting the different underlying kinetics.

Example 1

The first example involves hypothetical drug-activated channels with a kinetic scheme like that proposed for the acetylcholine receptor (Magleby and Stevens, 1972);

$$\mathrm{A} + \mathrm{R} \overset{\mathrm{K}}{\rightleftharpoons} \mathrm{AR} \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} \mathrm{AR}^* \tag{A}$$

where the state AR^* is the open state. The nonstationarity in this example will come from a time-dependence of the concentration $\mathrm{C_A}$ of the agonist A, of the form $C_A(t) = C_0\exp(-\lambda t)$. Equilibration between the states A + R and AR is assumed to be instantaneous, so we can write a scheme equivalent to (A) by condensing the two closed states into the single state (A,R),

$$(\mathrm{A},\mathrm{R}) \underset{\kappa(t)}{\overset{\alpha}{\rightleftharpoons}} \mathrm{AR}^*, \qquad (A')$$

where β has been replaced by the concentration-dependent rate

$$\kappa(t) = \frac{\beta C_{\mathrm{A}}(t)}{\mathrm{K} + C_{\mathrm{A}}(t)}$$

and the equation for the probability p of being in the open state is

$$\frac{\mathrm{d}p}{\mathrm{d}t} = \kappa(t)(1 - p) - \alpha p. \qquad (18)$$

For simplicity we assume the low-concentration limit: we let $C_0 \rightarrow 0$ and the number of channels $N \rightarrow \infty$ such that the number N_0 of channel opening events during an agonist pulse,

$$N_0 = N \int_0^\infty [1 - p(t)]\kappa(t)\mathrm{d}t \simeq \frac{N\beta C_0}{\lambda K},$$

remains a constant. In this limit, $\kappa(t) \simeq \beta\ C_{\mathrm{A}}(t)/K$ and p vanishes but the mean current I remains finite. Its time-course is obtained by using the initial condition $p(0) = 0$ in Eq. 18,

$$\begin{aligned} I(\mathrm{t}) &= Nip(t) \\ &= \frac{N_0 i\lambda}{\alpha - \lambda}[\exp(-\lambda t) - \exp(-\alpha t)], \end{aligned} \qquad (19)$$

and its time average over a long interval of length T is

$$\bar{I} \simeq \frac{1}{T}\int_0^\infty I(t)\mathrm{d}t = \frac{N_0 i}{\alpha T}.$$

The decay function $\phi(\tau)$ is computed[2], as the solution of Eq. 18 with the initial condition $p(0) = 1$, and is then made even in τ,

$$\phi(\tau) = \exp(-\alpha|\tau|). \qquad (20)$$

Membrane currents resulting from channels obeying scheme A were simulated with the parameter values $N_0 = 5{,}500$, $i = 1\,\mathrm{pA}$, $\alpha = 1\ \mathrm{ms}^{-1}$ and $\lambda = 0.1\ \mathrm{ms}^{-1}$. The record length was $T = 128$ ms. Rather than computing currents in a very large number N of channels, most of which never open, the simulation of a record started with the selection of a Poisson-distributed number of channels with mean N_0, each of which opens once. For each of these channels a pair of exponentially distributed random numbers with means λ^{-1} and α^{-1} were used to specify the

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[2] The time-dependence of the agonist concentration means that one of the transition rates in A' is time-dependent. The assumption of Eq. 2 nevertheless holds in this case because the low-concentration limit implies that each channel opens at most once. Thus the decay from the open state depends only on the invariant rate constant α.

delay before opening and the open time, respectively. Records obtained from this simulation are shown in Fig. 2 *A*.

Also shown in the figure is the power spectrum computed from differences in 50 pairs of records. Because p is vanishingly small, the power spectrum Eq. 10 should depend only on $\tilde{\Phi}(f)$. $\tilde{\Phi}$ and Φ should in turn differ negligibly because T is so much longer than the 1-ms time constant of ϕ. The prediction for the spectrum, calculated from Eqs. 12 and 13, is

$$i\bar{I}\Phi(f) = \frac{4i\bar{I}}{\alpha}\frac{1}{1 + (f/2\pi\alpha)^2}$$
$$= \frac{1.72 \times 10^{-19}}{1 + (f/159\ \text{Hz})^2}\ \text{A}^2/\text{Hz}. \tag{21}$$

The smooth curve shown in Fig. 2 *A* is the predicted Lorentzian function, which agrees well with the spectrum from the simulated currents. In the stationary case the form of the spectrum Eq. 21 would be exactly the same, with $\bar{I}$ then being equal to the steady current I.

Example 2

For this example, a scheme was chosen in which a substantial fraction of channels are opened, so that the correction term would be significant. The scheme is like that of voltage-gated channels with complete inactivation,

$$S_0 \underset{\alpha_m}{\overset{\beta_m}{\rightleftharpoons}} S_1^* \xrightarrow{\beta_h} S_2, \tag{B}$$

which has the functions

$$\phi(\tau) = a_1 \exp(-\lambda_1\tau) + a_2 \exp(-\lambda_2\tau)$$

$$I(t) = \frac{Ni\beta_m}{\lambda_1 - \lambda_2}[\exp(-\lambda_2 t) - \exp(-\lambda_1 t)]$$

$$\bar{I} = \frac{Ni\beta_m}{\lambda_1\lambda_2} \tag{22}$$

with the various parameters satisfying

$$a_1 = \frac{\lambda_1 - \alpha_m}{\lambda_1 - \lambda_2}$$

$$a_2 = 1 - a_1$$

$$\lambda_1 + \lambda_2 = \alpha_m + \beta_m + \beta_h$$

$$\lambda_1\lambda_2 = \alpha_m\beta_h.$$

The numerical values were chosen to be $N = 1{,}000$, $i = 1$ pA, $\alpha_m = 0.550\ \text{ms}^{-1}$, $\beta_m = 0.368\ \text{ms}^{-1}$ and $\beta_h = 0.182\ \text{ms}^{-1}$, giving the activation rate $\lambda_1 = 1\ \text{ms}^{-1}$ and the inactivation rate $\lambda_2 = 0.1\ \text{ms}^{-1}$, with $a_1 = a_2 = 0.5$. Fig. 1 *B* shows the functions p and ϕ from these parameter values. T was chosen to be 128 ms in the simulation, rather than 64 ms as in Fig. 1 *B*, to reduce the discrepancy between ϕ and $\tilde{\phi}$.

The simulation of the channel activity was similar to that in the previous example. Exponentially distributed random numbers were used for the dwell times in states s_0 and s_1, and another random number was used to choose between the two pathways for leaving s_1. The average spectral density was computed from 50 pairs of records as before, but then was corrected by adding the spectrum of one current record $y(t)$, scaled by the factor $1/N$. [An individual record y was a sufficiently good estimate of $I(t)$ for this purpose]. The corrected spectrum is expected to have two Lorentzian terms,

$$i\bar{I}\Phi(f) = 4i\bar{I}\frac{a_1}{\lambda_1}\frac{1}{1+(f/2\pi\lambda_1)^2}+\frac{a_2}{\lambda_2}\frac{1}{1+(f/2\pi\lambda_2)^2}$$

$$=\frac{8.6\times 10^{-25}}{1+(f/15.9\text{ Hz})^2}+\frac{8.6\times 10^{-26}}{1+(f/159\text{ Hz})^2}\text{ A}^2/\text{Hz}, \quad (23)$$

and is shown as a smooth curve in Fig. 2 *B*. The peak fraction of channels open was $p_{\max} \simeq 0.43$ in this example; the non-negligible values of p resulted in a correction term (dotted curve in the Figure) that contributed ~ 40% to the low-frequency component of the spectrum. If the rate constant α_h for the transition $s_2 \rightarrow s_1^*$ were made nonzero but small compared with β_h, inactivation would be incomplete. A spectrum obtained in the steady-state would then have the same form as Eq. 23 but a smaller amplitude, reflecting the small noninactivating current.

DISCUSSION

The main point of this paper is that power spectra obtained from nonstationary fluctuations from ionic channels can have a simple relationship to the conditional probability function p_{11}. Given certain assumptions that were discussed earlier, and in the limit of small p, the spectra from the nonstationary fluctuations are of exactly the same form as those from corresponding stationary fluctuations. For example, the same Lorentzian spectrum would have been obtained in Fig. 2 *A* if the agonist concentration had been held constant and stationary analysis had been used. The assumptions included the commonly made ones that channels are independently gated, identical, and have only two conductance levels. It was further assumed that the conditional probability function $p_{11}\,(t+\tau\,|\,t)$ is independent of t. If this assumption is not true, as is apparently the case for sodium channels in the node of Ranvier (Sigworth, 1981), the time average of p_{11} in Eq. 6 must be evaluated explicitly.

One special requirement of the nonstationary analysis is that the number of channels N or, equivalently, the probability of channels being open $p(t)$ must be known in order to correct the spectrum, as in Fig. 2 *B*. In many situations it will suffice to know that p is small (e.g. $p < 0.1$) since the relative size of the correction is on the order of p. Alternatively, N can be estimated by fitting a quadratic function to the variance-mean relationship (Sigworth, 1980) computed from a set of current records.

Another requirement is that the mean time-course must be accurately removed from the individual current records. Disaveraging pairs of records is a special case of the procedure in which the mean is computed from a group of n records and subtracted from each record in the group. As n increases, the excess variance in the spectrum decreases, but the sensitivity to drift also increases. More sophisticated disaveraging procedures that are insensitive to first and higher-order drifts with time could also be used. It is interesting to note, however, that a first-order drift, in which the magnitude of the current changes by a small constant factor from record to record, introduces an error of the same form as the correction term (see Eq. 14 of Sigworth, 1981) and is indistinguishable from a change in the value of N in Eq. 13. Such a drift also changes the apparent value of N as determined by the variance-mean relationship. These changes are compensatory, so that if N is determined from the variance-mean relationship of the same fluctuation records that are used for the power spectrum calculation, then a linear drift will result in no error in estimating the corrected spectrum.

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