ON THE TIME REVERSAL OF NOISE SIGNALS

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ABSTRACT This paper addresses the question of whether, and under what conditions, a noise trace changes its statistical properties when the time axis is reversed in direction. The autocorrelation function of the noise or its power spectrum cannot be used to identify the directionality of time in a noise signal since both are always the same for the signal and for its time reverse, regardless of the characteristics of the noise. However, the autocorrelation function can be generalized to represent the average of the products of powers of the signal at pairs of time instances separated by a given interval. If the powers are not the same for the first and second time instances, the generalized autocorrelation function can detect whether the statistical properties of a noise signal change upon the reversal of the direction of the time axis. We show that noise generated by systems that obey microscopic reversibility, i.e., that are at thermodynamic equilibrium, show the same statistical properties when evaluated forward and backward in time. A noise signal that does not demonstrate such time-reversal behavior discloses that the system that generates it is not at thermodynamic equilibrium. Several model examples are presented for illustration.

INTRODUCTION

A time-varying signal is superimposable on its time-reversed image, in which the direction of the time axis is reversed, if it is symmetrical about some line parallel to the signal coordinate. For such a signal, inversion of the algebraic sign of the time in the mathematical expression that describes the signal coupled with appropriate translation along the time axis (i.e. substitution of $-t + \theta$ for t, θ being an adjustable parameter that fixes the origin of the time axis) generates the same signal. We shall refer to such a signal as one that obeys time reversal. Obviously, not all signals obey time reversal. Thus, a Gaussian signal, $\exp[-(t - a)^2]$, obeys time reversal, since it is superimposable on $\exp[-(-t + a)^2]$ (θ being in this case $2a$), whereas a nerve action potential, for example, does not obey time reversal.

Here we shall examine the time-reversal properties of noise traces. Since such traces hardly ever reproduce themselves on consecutive runs even without time reversal, due to their stochastic nature, it is of course of no consequence to compare them directly upon time reversal. Rather, one should compare the behavior of appropriate measures of their statistical properties upon time reversal, appropriate measures being those that depend on the direction of the time arrow. The autocorrelation function of a signal or its power spectrum do not fulfill this requirement, since they are always the same for any signal and its time reverse. We shall suggest some statistical properties of signals that do change upon time reversal, and then use these properties to investigate the time-reversal behavior of noise. A few model cases will then be presented for illustration.

AUTOCORRELATION FUNCTIONS THAT CHANGE UPON TIME REVERSAL

Let $f(t)$ be a time-dependent signal. The autocorrelation function, $G(\tau)$, of $f(t)$ is approximated by

$$G(\tau) = \frac{1}{(T_2 - \tau) - T_1} \int_{T_1}^{T_2 - \tau} f(t) f(t + \tau) \mathrm{d}t, \qquad (1)$$

where T_1 and T_2 define the range of time over which information about the signal is available. Eq. 2 readily shows that the autocorrelation function, $G_r(\tau)$, of the time-reversed function, $f_r(t)[=f(-t)]$, is identical to $G(\tau)$

$$\begin{aligned} G_r(\tau) &= \frac{1}{T_2 - T_1 - \tau} \int_{-T_2}^{-T_1 - \tau} f_r(t) f_r(t + \tau) \mathrm{d}t \\ &= \frac{1}{T_2 - T_1 - \tau} \int_{-T_2}^{-T_1 - \tau} f(-t) f[-(t + \tau)] \mathrm{d}t \\ &= -\frac{1}{T_2 - T_1 - \tau} \int_{T_2 - \tau}^{T_1} f(\xi + \tau) f(\xi) \mathrm{d}\xi \\ &= \frac{1}{T_2 - T_1 - \tau} \int_{T_1}^{T_2 - \tau} f(\xi) f(\xi + \tau) \mathrm{d}\xi = G(\tau), \end{aligned} \qquad (2)$$

where $\xi = -(t + \tau)$. The limits of integration in Eq. 2 with respect to t can be deduced readily by inspection of Fig. 1, in which an example of $f(t)$ and $f_r(t)$ is presented.

The autocorrelation functions of any signal, and of the corresponding time-reversed signal, are thus identical. It may be noted that such an identity was noted before for a special case (Sigworth, 1981). Also it follows that the power spectra of any signal and the corresponding time-reversed signal are identical, since the power spectra are

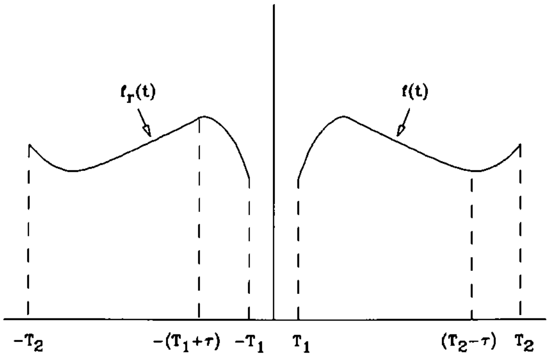

FIGURE 1 An example of $f(t)$ and $f_r(t)$.

related to the autocorrelation functions by a Fourier transform. The outcome of Eq. 2 is not surprising since $G(\tau)$ is the average of the product of the values of $f(t)$ at intervals of t separated τ apart, regardless of the direction of the time axis. Directionality in time can, however, become a contributing factor if one uses autocorrelation functions $G^{\alpha,\beta}(\tau)$ of the following type:

$$G^{\alpha,\beta}(\tau) = \frac{1}{(T_2 - \tau) - T_1} \int_{T_1}^{T_2-\tau} f^{\alpha}(t) f^{\beta}(t + \tau)\mathrm{d}t, \quad (3)$$

where $f^{\alpha}(t)$ and $f^{\beta}(t)$ are $f(t)$ raised to the power α and β, respectively. If one chooses $\alpha \neq \beta$, the autocorrelation function $G_r^{\alpha,\beta}(\tau)$ of the time-reversed function $f_r(t)$ is not necessarily equal to $G^{\alpha,\beta}(\tau)$. Thus,

$$\begin{aligned}
G_r^{\alpha,\beta}(\tau) &= \frac{1}{T_2 - T_1 - \tau} \int_{-T_2}^{-T_1-\tau} f_r^{\alpha}(t) f_r^{\beta}(t + \tau)\mathrm{d}t \\
&= \frac{1}{T_2 - T_1 - \tau} \int_{-T_2}^{-T_1-\tau} f^{\alpha}(-t) f^{\beta}[-(t + \tau)]\mathrm{d}t \\
&= -\frac{1}{T_2 - T_1 - \tau} \int_{T_2-\tau}^{T_1} f^{\alpha}(\xi + \tau) f^{\beta}(\xi)\mathrm{d}\xi \\
&= \frac{1}{T_2 - T_1 - \tau} \int_{T_1}^{T_2-\tau} f^{\beta}(\xi) f^{\alpha}(\xi + \tau)\mathrm{d}\xi = G^{\beta,\alpha}(\tau). \quad (4)
\end{aligned}$$

It follows that $G_r^{\alpha,\beta}(\tau)$ is equal to $G^{\beta,\alpha}(\tau)$ but not necessarily to $G^{\alpha,\beta}(\tau)$, except when $f(t)$ obeys time reversal, i.e. $f_r(t) = f(-t) = f(t + \theta)$. In such a case,

$$\begin{aligned}
G_r^{\alpha,\beta}(\tau) &= \frac{1}{T_2 - T_1 - \tau} \int_{-T_2}^{-T_1-\tau} f_r^{\alpha}(t) f_r^{\beta}(t + \tau)\mathrm{d}t \\
&= \frac{1}{T_2 - T_1 - \tau} \int_{-T_2}^{-T_1-\tau} f^{\alpha}(-t) f^{\beta}[-(t + \tau)]\mathrm{d}t \\
&= \frac{1}{T_2 - T_1 - \tau} \int_{-T_2}^{-T_1-\tau} f^{\alpha}(t + \theta) f^{\beta}(t + \theta + \tau)\mathrm{d}t \\
&= \frac{1}{T_2 - T_1 - \tau} \int_{-T_2+\theta}^{-T_1-\theta-\tau} f^{\alpha}(\eta) f^{\beta}(\eta + \tau)\mathrm{d}\eta \\
&= \frac{1}{T_2 - T_1 - \tau} \int_{T_1}^{T_2-\tau} f^{\alpha}(\eta) f^{\beta}(\eta + \tau)\mathrm{d}\eta = G^{\alpha,\beta}(\tau), \quad (5)
\end{aligned}$$

where $\eta = t + \theta$. Inspection of Fig. 2 shows that $-T_2 + \theta = T_1$, and $-T_1 + \theta = T_2$, which accounts for the changes in the limits of integration in Eq. 5. Note that an alternate way for expressing $G_r^{\alpha,\beta}(\tau)$ is $G^{\alpha,\beta}(-\tau)$ defined by Eq. 6

$$\begin{aligned}
G^{\alpha,\beta}(-\tau) &= \frac{1}{T_2 - T_1 - \tau} \int_{T_1+\tau}^{T_2} f^{\alpha}(t) f^{\beta}(t - \tau)\mathrm{d}t \\
&= -\frac{1}{T_2 - T_1 - \tau} \int_{-T_1-\tau}^{-T_2} f^{\alpha}(-\phi) f^{\beta}(-\phi-\tau)\mathrm{d}\phi \\
&= \frac{1}{T_2 - T_1 - \tau} \int_{-T_2}^{-T_1-\tau} f_r^{\alpha}(\phi) f_r^{\beta}(\phi + \tau)\mathrm{d}\phi \\
&= G_r^{\alpha,\beta}(\tau), \quad (6)
\end{aligned}$$

where $\phi = -t$.

Autocorrelation functions of $f(t)$ of the type $G^{\alpha,\beta}(\tau)$ may thus serve as indicators to show whether $f(t)$ obeys time reversal or not. If it does, $G^{\alpha,\beta}(\tau) = G_r^{\alpha,\beta}(\tau)$. An equivalent way of ascertaining time reversal is by verification that the equality $G^{\alpha,\beta}(\tau) = G^{\beta,\alpha}(\tau)$ holds, since $G_r^{\alpha,\beta}(\tau) = G^{\beta,\alpha}(\tau)$ (see Eq. 4). If $f(t)$ does not obey reversal, these equalities are not obeyed as a rule (although they may be obeyed by coincidence in special cases). Whereas these criteria for time reversal of a signal are probably superfluous for deterministic signals, they might be useful for noise signals, which occur stochastically, and whose symmetry properties, specifically time reversal, may easily elude visual inspection.

The simplest useful autocorrelation functions $G^{\alpha,\beta}(\tau)$, for which $\alpha \neq \beta$ are $G^{1,2}(\tau)$ and $G^{2,1}(\tau)$. However, for even values of α and β we miss information as to whether the correlations in $f(t)$ are positive or negative. We shall generally use $G^{1,3}(\tau)$ for the sake of illustration, but shall sometimes allude to other autocorrelation functions as well. It may be noted that the autocorrelation function $G^{1,3}(\tau)(N)$ of the noise produced by N statistically independent similar systems is related to the autocorrelation function $G^{1,3}(\tau)$ of the noise produced by a single system by the following expression:

$$G^{1,3}(\tau)(N) = N \cdot G^{1,3}(\tau) + N(N-1) \cdot \langle f(t) \rangle \cdot \langle f^3(t) \rangle, \quad (7)$$

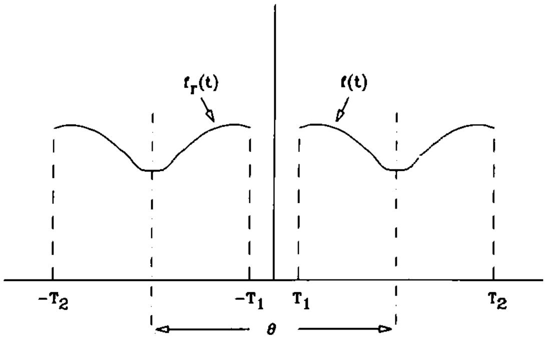

FIGURE 2 An example of a signal that obeys time reversal. θ is twice the distance of the axis of symmetry of the signal from the origin.

where $\langle f(t)\rangle$ and $\langle f^3(t)\rangle$ are the time averages of $f(t)$ and $f^3(t)$, respectively. Thus, for the autocorrelation function $G_\Delta^{1,3}(\tau)$ of the fluctuations of $f(t)$ about the average of $f(t)$, i.e., for fluctuations of $\Delta f(t) = f(t) - \langle f(t)\rangle$ the second term on the right-hand side of Eq. 7 vanishes, and one obtains,

$$G_\Delta^{1,3}(\tau)(N) = N \cdot G_\Delta^{1,3}(\tau). \tag{8}$$

In the examples we will present we use the autocorrelations of the fluctuations of $\Delta f(t)$. Note that Eq. 8 holds also for other values of α and β, provided that either α or β is equal to unity. The usefulness of the relation expressed in Eq. 8 makes the choice of unity for α or β very convenient. It is also worth noting that $G_\Delta^{\alpha,\beta}(\infty)$ of noise equals to $\langle[\Delta f(t)]^\alpha\rangle \cdot \langle[\Delta f(t)]^\beta\rangle$, since $f(t)$ assumably loses all correlations over infinite time. Thus $G_\Delta^{\alpha,\beta}(\infty)$ vanishes only if either α or β is unity.

It is pertinent to note that functions of the type $G^{\alpha,\beta}(\tau)$, as defined by Eq. 3, are not the only ones that may depend on the direction of the time axis. As a matter of fact, any correlation function of the type $\langle Y_1[f(t)]Y_2[f(t+\tau)]\rangle$, where Y_1 and Y_2 are two different functions of the amplitudes of $f(t)$ at times t and $t+\tau$, respectively, will behave similarly. An example will be presented below (see Fig. 4 *D*).

TIME REVERSAL OF NOISE SIGNALS

Stationary Noise

It was previously shown (Neher and Stevens, 1977; Haas and Steinberg, 1984) that the autocorrelation function $G^{1,1}(\tau)$ of a stationary noise signal (i.e., one whose statistical properties do not vary with time) can be related by the following expression to the elementary processes that take place in the system that produces the noise:

$$G^{1,1}(\tau) = \sum_{i=1}^{s} F_iS_i \sum_{j=1}^{s} S_j p(j/i;\tau), \tag{9}$$

where i and j ($i = 1, 2, \ldots, s$; $k = 1, 2, \ldots, s$) denote the various states in which the system can be, the total number of states being s; S_i and S_j are the amplitudes of the measured signal when the system is in state i and state j, respectively; F_i is the probability of finding the system in state i (i.e., it is the fraction of time that the system spends in state i); and $p(j/i,\tau)$ is the probability for a system that is in state i to be transformed into state j during a time interval τ. An implicit assumption in the above equation is that $p(j/i,\tau)$ is a function of the time interval τ alone, and does not depend on the history of the system, i.e., that the evolution of the system is Markovian, which is usually assumed to be the case in chemical kinetics. The physical nature of the measured signal is not relevant to the analysis; it may be, for example, fluorescence light intensity emitted by molecules that fluctuate in conformation, provided the emitted light intensity is different for the different conformations (Haas and Steinberg, 1984); or it may be the electric current flowing through the surface of an excitable cell via channels that fluctuate in their conductance state (Neher and Stevens, 1977; Sakmann and Neher, 1983). The rationale that leads to Eq. 6 is as follows: We divide up the time axis into segments, each of which denote time intervals where the system is in given state i (i can take up the values $1, 2, \ldots, s$). The fraction of time in which the system is in state i is F_i. The system starting out in state i has the probability $p(j/i;\tau)$ of converting into state j ($j = 1, 2, \ldots, s$) after a time interval τ. Thus, the contribution to $G^{1,1}(\tau)$ of the time durations in which the system starts out in a specified state i is $F_iS_i\Sigma_{j=1}^{s}S_jp(j/i;\tau)$. To obtain the whole function $G^{1,1}(\tau)$ one has to sum up the contributions of the various i initial states, which leads to the expression for $G^{1,1}(\tau)$ in Eq. 9.

In an analogous way one can show that the corresponding expression for $G^{\alpha,\beta}(\tau)$ is

$$G^{\alpha,\beta}(\tau) = \frac{1}{T-\tau}\int_o^T f^\alpha(t)f^\beta(t+\tau)\mathrm{d}t = \sum_{i=1}^{s} F_iS_i^\alpha \sum_{j=1}^{s} S_j^\beta p(j/i;\tau) \tag{10}$$

and

$$G_\Delta^{\alpha,\beta}(\tau) = \frac{1}{T-\tau}\int_o^T [f(t) - \langle f(t)\rangle]^\alpha[f(t+\tau) - \langle f(t)\rangle]^\beta \mathrm{d}t = \sum_{i=1}^{s} F_i(S_i - \bar{S})^\alpha \sum_{i=1}^{s} (S_j - \bar{S})^\beta p(j/i;\tau), \tag{11}$$

where $\bar{S}$ is the average signal height, $\bar{S} = \Sigma_{i=1}^{s}F_iS_i$. The following considerations for $G^{\alpha,\beta}(\tau)$ apply to $G_\Delta^{\alpha,\beta}(\tau)$ as well.

In the expression for the time-reversed autocorrelation function, $G_r^{\alpha,\beta}(\tau)$, the parameters F_i, S_i, and S_j assume, of course, the same values as in that for $G^{\alpha,\beta}(\tau)$; however, $p(j/i;\tau)$ should be replaced by $p(j/i;-\tau)$, the latter defining the probability that a system found in state i was at time τ earlier in state j. Thus,

$$G_r^{\alpha,\beta}(\tau) = \sum_{i=1}^{s} F_iS_i^\alpha \sum_{j=1}^{s} S_j^\beta p(j/i;-\tau). \tag{12}$$

A sufficient condition for the time reversal of the signal, i.e. $G^{\alpha,\beta}(\tau) = G_r^{\alpha,\beta}(\tau)$, is that $p(j/i;\tau) = p(j/i;-\tau)$. It should be noted that this is not a necessary condition for obtaining $G_r^{\alpha,\beta}(\tau) = G^{\alpha,\beta}(\tau)$. For example, the trivial case in which all S_i ($i = 1, 2, \ldots, s$) are equal to one another will ensure that $G_r^{\alpha,\beta}(\tau) = G^{\alpha,\beta}(\tau)$; such a case obviously does not contain the necessary information about the time reversal of $p(j/i;\tau)$. On the other hand, if $G_r^{\alpha,\beta}(\tau)$ is found in a specific experiment to be different than $G^{\alpha,\beta}(\tau)$ it follows that $p(j/i;\tau) \neq p(j/i;-\tau)$. It is therefore of interest to examine the significance of the equality or inequality of $p(j/i;\tau)$ with its time reverse, $p(j/i;-\tau)$.

Let us take an ensemble of identical systems in which we have the various systems in the various states $i = 1, 2, \ldots, s$ at the steady-state distribution at time $t = -\tau$. Obviously, if the ensemble is very large, it will also be characterized by the same steady-state distribution also at $t = 0$, with negligible fluctuations. The fractional numbers of systems in states $1, 2, \ldots$ and s are given by $F_1, F_2, \ldots,$ and F_s, respectively. The probability $p(j/i; -\tau)$ that a system presently in state i was in state j at time τ earlier is given by the number of systems that were transformed from state j into state i in this time interval, i.e., $F_j \cdot p(i/j; \tau)$, divided by the total number of systems presently in state i, regardless of their state τ units of time earlier, i.e., $\Sigma_{k=1}^{s} F_k \cdot p(i/k; \tau)$. Thus, (Feller, 1968)

$$p(j/i; -\tau) = \frac{F_j p(i/j; \tau)}{\sum_{k=1}^{s} F_k p(i/k; \tau)} = \frac{F_j p(i/j; \tau)}{F_i}. \tag{13}$$

The second equality in Eq. 13 follows from the fact that for a system that is in steady state $\Sigma_{k=1}^{s} F_k p(i/k; \tau) = F_i$, since each represents the fractional number of systems in the ensemble that are in state i.

If the various processes in the system that are responsible for the noise signal obey detailed balance, i.e., microscopic reversibility, the following relationship holds:

$$F_i p(j/i; \tau) = F_j p(i/j; \tau). \tag{14}$$

Combining Eqs. 13 and 14, one obtains

$$p(j/i; -\tau) = p(j/i; \tau). \tag{15}$$

It should be stressed that Eq. 15 holds only for systems with elementary processes that obey detailed balance. This requires that the system be in thermodynamic equilibrium (Tolman, 1938; Katchalsky and Curran, 1965). Conversely, if $p(j/i; -\tau) \neq p(j/i; \tau)$ for a given system, then it is not in thermodynamic equilibrium. Thus we may reach the interesting conclusions that if one observes a noise signal for which $G^{\alpha,\beta}(\tau)$ ($\alpha \neq \beta$) is different for the signal and for its time reverse, then $p(j/i; -\tau) \neq p(j/i; \tau)$ (compare Eqs. 10 and 12), and hence that the system producing the noise is not in thermodynamic equilibrium. However, as stated above, and as will be discussed later, the reverse is not necessarily true, i.e., noise signals for which $G^{\alpha,\beta}(\tau) = G_r^{\alpha,\beta}(\tau)$, may still originate from systems for which $p(j/i; -\tau) \neq p(j/i; \tau)$, systems that are not in thermodynamic equilibrium.

Nonstationary Noise

The above considerations referred to stationary noise signals, i.e., such signals for which the various parameters that define them do not change during the sampling time. Whereas most experiments that derive information from the behavior of noise are designed for stationary conditions, it is sometimes advantageous to study noise under nonstationary conditions (Sigworth, 1980, 1981*a*, 1981b; Conti et al., 1980; Hess and Tsien, 1984). The systems producing such noise are of course not at thermodynamic equilibrium and therefore the issues discussed above are relevant to nonstationary noise. The definitions of the correlation functions are, however, somewhat modified to suit the nonstationary conditions.

Since the statistical parameters change during the sampling time, one does not evaluate the correlation functions by averaging along the time axis. Rather, correlations are defined between signal amplitudes at pairs of instances t_1 and t_2 after the trigger that initiates the nonstationary conditions. Thus the autocorrelation function in this case is a function of two variables, t_1 and t_2, and is defined as follows (Sigworth, 1981*a*):

$$G_\Delta(t_1, t_2) = \langle [f(t_1) - \overline{f(t_1)}][f(t_2) - \overline{f(t_2)}] \rangle, \tag{16}$$

where $\overline{f(t_1)}$ and $\overline{f(t_2)}$ are the averages of $f(t_1)$ and $f(t_2)$, respectively, obtained after many triggering events that start the nonstationary signal. The autocorrelation function of the reverse sequence of time is

$$G_\Delta(t_2, t_1) = \langle [f(t_2) - \overline{f(t_2)}[f(t_1) - \overline{f(t_1)}] \rangle \tag{17}$$

which is obviously identical to $G_\Delta(t_1, t_2)$. However, the generalized autocorrelation function $G_\Delta^{\alpha,\beta}(t_1, t_2)$, defined in analogy to Eq. 3,

$$G_\Delta^{\alpha,\beta}(t_1, t_2) = \langle [f(t_1) - \overline{f(t_1)}]^\alpha [f(t_2) - \overline{f(t_2)}]^\beta \rangle \tag{18}$$

is not necessarily identical to the corresponding autocorrelation function of the reversed time sequence

$$G_\Delta^{\alpha,\beta}(t_2, t_1) = \langle [f(t_2) - \overline{f(t_2)}]^\alpha [f(t_1) - \overline{f(t_1)}]^\beta \rangle \tag{19}$$

if $\alpha \neq \beta$, and may thus serve as a measure of the time-reversal properties of the noise. Comparing Eqs. 18 and 19 one obtains,

$$G_\Delta^{\alpha,\beta}(t_2, t_1) = G_\Delta^{\beta,\alpha}(t_1, t_2). \tag{20}$$

The autocorrelation function $G_\Delta^{1,1}(t_1, t_2)$ has been expressed for nonstationary noise by Sigworth (1981) in terms of the kinetics of the elementary processes in the noise-generating system. One may formulate an expression for $G_\Delta^{\alpha,\beta}(t_1, t_2)$ in a similar way

$$G_\Delta^{\alpha,\beta}(t_1, t_2) = \sum_{i=1}^{s} F_i(t_1) [S_i - \overline{S(t_1)}]^\alpha \sum_{j=1}^{s} [S_j - \overline{S(t_2)}]^\beta p(j, t_2/i, t_1), \tag{21}$$

where $\overline{S(t_1)}$ and $\overline{S(t_2)}$ are the average signals at time t_1 and t_2, respectively. $F_i(t_1)$ is the probability of finding the system in state i at time t_1, and $p(j, t_2/i, t_1)$ is the probability of finding the system in state j at time t_2, given that it was in state i at time t_1. All other symbols have the same significance as in Eq. 9. The rationale for Eq. 21 is analogous to that for Eq. 6 (see above). The corresponding autocorrelation function for the time-reversed sequence may similarly be written as

$$G_{\Delta}^{\alpha,\beta}(t_2, t_1)$$

$$=\sum_{i=1}^{s} F_i(t_2)[S_i - \overline{S(t_2)}]^{\alpha} \sum_{j=1}^{s} [S_j - \overline{S(t_2)}]^{\beta} p(j, t_1/i, t_2), \quad (22)$$

$p(j, t_1/i, t_2)$ being the probability that the system was at time t_1 in state j, given that it is in state i at t_2. As with the derivation of Eq. 13 for this probability one readily obtains

$$p(j, t_1/i, t_2) = \frac{F_j(t_1)p(i, t_2/j, t_1)}{\sum_{k=1}^{s} F_k(t_1)p(i, t_2/k, t_1)}$$

$$= \frac{F_j(t_1)p(i, t_2/j, t_1)}{F_i(t_2)}. \quad (23)$$

Under nonstationary conditions, at least some of the values of $F_k(t)$ $(k = 1, 2, \ldots, s)$ change, by definition. Thus, at least for some $F_i(t_1)$ and $F_j(t_1)$ the following inequality holds:

$$F_j(t_1)p(i, t_2/j, t_1) \neq F_i(t_1)p(j, t_2/i, t_1), \quad (24)$$

the left-hand side denoting the interconversion of state j into state i in the time range between t_1 and t_2, and the right hand side denoting the reverse interconversion. Thus,

$$p(j, t_1/i, t_2) \neq p(j, t_2/i, t_1) \quad (25)$$

at least for some values of i and j. A comparison of Eqs. 21 and 22, in conjunction with the inequality of Eq. 25, shows that for nonstationary noise

$$G_{\Delta}^{\alpha,\beta}(t_1, t_2) \neq G_{\Delta}^{\alpha,\beta}(t_2, t_1), \quad (26)$$

which, expressed otherwise (see Eq. 20), yields

$$G_{\Delta}^{\alpha,\beta}(t_1, t_2) \neq G_{\Delta}^{\beta,\alpha}(t_1, t_2). \quad (27)$$

It is of interest to note that whereas a two-state system should exhibit microscopic reversibility at ready state, and hence the noise it generates should exhibit time reversal, this is not the case under nonstationary conditions. Then the two states are not in detailed balance and the noise generated does not obey time reversal.

ILLUSTRATION BY SIMPLE MODEL SYSTEMS

Stationary Noise

The effect of the thermodynamic state of the noise-producing system on the statistical time-reversal properties of the generated noise will be demonstrated by two simple model systems that can be treated analytically. The systems are assumably at steady state; however, in one case, Case I, the system assumably obeys microscopic reversibility, i.e., is at thermodynamic equilibrium, whereas in the other case (Case II) it does not obey microscopic reversibility. In both cases the systems are comprised of three interconverting species, A, B, and C, as shown in Fig. 3. In Case I the thermodynamic equilibrium requirement for the detailed balance $k_{AB}[A]_a = k_{BA}[B]_a$, $k_{BC}[B]_a = k_{CB}[C]_a$, and $k_{AC}[A]_a = k_{CA}[C]_a$ is obeyed, where $[A]_a$, $[B]_a$, and $[C]_a$ denote the average concentrations of the corresponding species at steady state. (The definition of the various rate constants is given in Fig. 3). In contrast, such detailed balance is not obeyed in Case II. One can envisage a variety of biochemical processes that can be modeled by Case II. For example, an enzyme molecule in state A may reversibly bind a substrate molecule and be converted in the process into state B, which in turn forms a covalent intermediate represented by C. Hydrolysis of the covalent intermediate C regenerates the enzyme in state A. If the various states of the enzyme can be monitored by some physical measure (e.g., fluorescence intensity), which is different for the different states [e.g., due to different extents of loss of intensity resulting from energy transfer (Stryer, 1968; Haas et al., 1975)], the noise of the signal will be one that is generated by a system that does not obey microscopic reversibility. A, B, and C may similarly represent various states, of different electrical conductivity, of an ionic channel that is capable of being covalently modified, e.g., by phosphorylation. In this case the measured noise is that of electric current if the voltage is clamped.

The kinetic behavior of the species A, B, and C can be described by a set of three differential equations. Note, however, that the quantities of the three species are not independent of one another, their sum being constant and equal to their total quantity $[T]$ in the system. It is therefore sufficient to describe the kinetic behavior of the system by two differential equations. For the sake of illustration let us assume that in the model systems depicted in Fig. 3 all rate constants assume the value of unity. For Case I we thus obtain

$$\mathrm{d}[A]/\mathrm{d}t = -3[A] + [T]$$
$$\mathrm{d}[B]/\mathrm{d}t = -3[B] + [T], \quad (28)$$

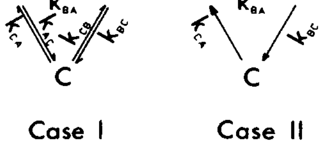

FIGURE 3 Illustration of a system that can obey detailed balance, Case I, and of a system that does not obey detailed balance (i.e., that is not at thermodynamic equilibrium), Case II.

which yields the solutions

$$[A] = ([A]_o - [T]/3)\exp(-3t) + [T]/3$$
$$[B] = ([B]_o - [T]/3)\exp(-3t) + [T]/3$$
$$[C] = ([C]_o - [T]/3)\exp(-3t) + [T]/3. \qquad (29)$$

The subscript 0 denotes the initial values of the respective concentrations at $t = 0$. The sets of probabilities $p(j/i;\tau)$ necessary for the evaluation of $G^{\alpha,\beta}(\tau)$ (see Eqs. 10 and 11) are obtained from Eq. 29 by setting $[T]$, as well as the initial value of the ith species, each equal to unity. Thus,

$$p(j/i;\tau) = (\delta_i^j - 1/3)\exp(-3\tau) + 1/3, \qquad (30)$$

where $\delta_i^j = 1$ for $i = j$ and vanishes otherwise. Inserting the expressions thus obtained for $p(j/i;\tau)$ into Eq. 10 one obtains

$$\begin{aligned} G^{\alpha,\beta}(\tau) &= \sum_i F_i S_i^\alpha \sum_j S_j^\beta p(j/i;\tau) \\ &= (1/9)\,\{(S_A^{\alpha+\beta} + S_B^{\alpha+\beta} + S_C^{\alpha+\beta})[2\exp(-3\tau) + 1] \\ &\quad + [(S_A^\alpha S_B^\beta + S_A^\beta S_B^\alpha) + (S_A^\alpha S_C^\beta + S_A^\beta S_C^\alpha) \\ &\quad + (S_B^\alpha S_C^\beta + S_B^\beta S_C^\alpha)] \quad [1 - \exp(-3\tau)]\}. \qquad (31) \end{aligned}$$

Obviously, interchange of α and β yields the same expression also when $\alpha \neq \beta$. The noise produced by the scheme represented as Case I (Fig. 3) exhibits time-reversal properties, as expected for a system that fulfills microscopic reversibility. Let us take as a specific example the following parameters: $\alpha = 1$, $\beta = 3$, $S_A = 0$, $S_B = 2$, and $S_C = 1$. The corresponding values for $S_A - \overline{S}$, $S_B - \overline{S}$, and $S_C - \overline{S}$ are -1, 1, and 0, respectively ($\overline{S} = \Sigma_i F_i S_i = 1$). Insertion in Eq. 32 with the modification required by Eq. 11 yields

$$G_\Delta^{1,3}(\tau) = G_\Delta^{3,1}(\tau) = (2/3)\exp(-3\tau). \qquad (32)$$

Let us now turn to Case II, which does not obey microscopic reversibility. The following differential equations describe the kinetic behavior in this case.

$$\mathrm{d}[A]/\mathrm{d}t = -2[A] + [T]$$
$$\mathrm{d}[B]/\mathrm{d}t = [A] - 2[B] \qquad (33)$$

remembering that in our examples we adopted the magnitude of unity for all rate constants. The values of F_i correspond to the steady-state concentrations of A, B, and C (taking $[T] = 1$), which can readily be seen to be 1/2, 1/4, and 1/4, respectively. The solutions of the above equations are

$$\begin{aligned} [A] &= ([A]_o - [T]/2)\exp(-2t) + [T]/2 \\ [B] &= ([A]_o - [T]/2)\cdot t\cdot\exp(-2t) \\ &\quad + ([B]_o - [T]/4)\exp(-2t) + [T]/4 \\ [C] &= [T] - [A] - [B]. \qquad (34) \end{aligned}$$

The set of probabilities $p(j/i;\tau)$ is obtained by setting $[T] = 1$ and the initial value of the ith species equal to unity. Upon substitution in Eq. 10 and rearrangement one obtains for $G^{\alpha,\beta}(\tau)$,

$$\begin{aligned} G^{\alpha,\beta}(\tau) &= (1/16)\,\{[2(-S_B^{\alpha+\beta} + S_C^{\alpha+\beta}) + 4(S_A^\alpha S_B^\beta - S_A^\alpha S_C^\beta) \\ &\quad + 2(S_B^\alpha S_C^\beta - S_B^\beta S_C^\alpha)]\cdot\tau\cdot\exp(-2\tau) \\ &\quad + 4S_A^{\alpha+\beta}[1 + \exp(-2\tau)] + (S_B^{\alpha+\beta} + S_C^{\alpha+\beta})[1 \\ &\quad + 3\exp(-2\tau)] + [2(S_A^\alpha S_B^\beta + S_A^\beta S_B^\alpha) + 2(S_A^\alpha S_C^\beta \\ &\quad + S_A^\beta S_C^\alpha) + (S_B^\alpha S_C^\beta + S_B^\beta S_C^\alpha)][1 - \exp(-2\tau)]\}. \qquad (35) \end{aligned}$$

Obviously, if $\alpha \neq \beta$ and $S_A \neq S_B \neq S_C$ the interchange of indices in $G^{\alpha,\beta}(\tau)$ yields a different expression for the autocorrelation function, which manifests itself in different coefficients of the function $\tau \cdot \exp(-2\tau)$ in $G^{\alpha,\beta}(\tau)$ and $G^{\beta,\alpha}(\tau)$. Thus, the noise produced by the model system described as Case II in Fig. 3 does not exhibit time reversal, as expected from the fact that this system does not fulfill microscopic reversibility and is thus not in thermodynamic equilibium, though it may be in a steady-state condition. It is worth noting that if the specific intensities of the signals produced by A, B, and C, i.e. S_A, S_B, and S_C, are not all different from one another the noise produced by the fluctuations of the system will not reveal the violation of time reversal. In such a case the noise does not contain enough information to disclose the lack of thermodynamic equilibrium in the noise-generating system.

As a specific example let us choose the following values for the signal intensities of A, B, and C: $S_A = 0$, $S_B = 2$, and $S_C = 1$. The corresponding values for $S_A - \overline{S}$, $S_B - \overline{S}$, and $S_C - \overline{S}$ are $-3/4$, 5/4, and 1/4, respectively. Fig. 4 graphically demonstrates some examples of $G_\Delta^{\alpha,\beta}(\tau)$ and their corresponding reverse functions evaluated by insertion of the above parameters in Eq. 35 together with the modification required by Eq. 11. Clearly, for the chosen values of the kinetic parameters and the intensities of the signals produced by the various interconverting species, the various pairs of correlation functions $G_\Delta^{\alpha,\beta}(\tau)$ and their corresponding reverse functions do not coincide for the model system shown in Fig. 3, Case II. The degree of discrepancy between $G_\Delta^{\alpha,\beta}(\tau)$ and the reverse functions depends on the specific values of α and β. Thus it may be advisable in practice to try various values of these parameters. Fig. 4 *C* illustrates the fact that if α or β is not unity, $G_\Delta^{\alpha,\beta}(\tau)$ does not approach zero at infinite time. In Fig. 4 *D* autocorrelation functions of the general type $\langle Y_1[f(t)]Y_2[f(t+\tau)]\rangle$ are illustrated, with $Y_1(x) = x$ and $Y_2(x) = \exp(x) - 1 - x$, and vice versa. It may be noted that $\exp(x) - 1 - x = x^2/2! + x^3/3! + \ldots$. These autocorrelation functions are thus linear combinations of the functions $G_\Delta^{1,\beta}(\tau)$ and $G_\Delta^{\beta,1}(\tau)$ with $\beta = 2, 3, \ldots, \infty$.

Nonstationary Noise

A very simple fluctuating, noise-generating, system is the following:

$$A \underset{k_{BA}}{\overset{k_{AB}}{\rightleftharpoons}} B, \qquad (36)$$

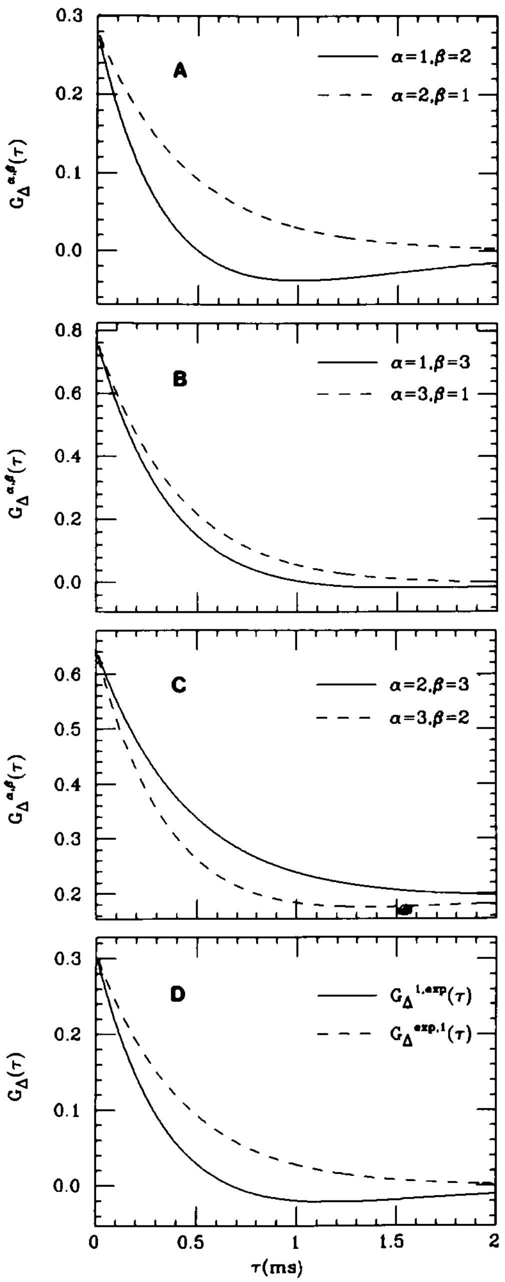

FIGURE 4 A comparison between some autocorrelation functions and the corresponding reverse autocorrelations functions of the noise produced by a system which is at steady state but not at equilibrium. The noise-producing system is the one shown as Case II, Fig. 3. The values of the parameters used to evaluate the autocorrelation functions presented are: $k_{AB} = k_{BA} = k_{BC} = k_{CA} = 1\ \mathrm{ms}^{-1}$, $S_A = 0$, $S_B = 2$, $S_C = 1$. In A, B, and C, $G_\Delta^{\alpha,\beta}(\tau)$ have the form defined by Eq. 3 with $f(t)$ replaced by $f(t) - \langle f(t) \rangle$. In D, $G_\Delta^{1,\exp}(\tau) = \langle \Delta f(t) \cdot [\exp \Delta f(t+\tau) - 1 - \Delta f(t+\tau)] \rangle$, and $G_\Delta^{\exp,1}(\tau) = \langle [\exp \Delta f(t) - 1 - \Delta f(t)] \cdot \Delta f(t+\tau) \rangle$. For details, see text.

provided A and B produce different amplitudes of the measured signal (fluorescence intensity, ionic currents, etc., as the case may be). Under steady-state conditions such a system necessarily obeys detailed balance, since the concentrations of A and B are constant; hence the noise that the system produces should demonstrate time reversal. However, under nonstationary conditions, when the system has been perturbed out of equilibrium (e.g., by stepping the membrane potential of an excitable cell in electric noise experiments), $[A]$ and $[B]$ change with time, and the noise during the relaxation process is expected to violate time-reversal.

As a specific example, let us assume that at zero time the system is in state A, i.e., $F_A(0) = 1$, and that $k_{AB} = 2k_{BA}$. One can obtain the expressions for $F_A(t_1)$, $F_B(t_1)$, and the set of probabilities $p(j, t_2/i, t_1)$ by solving the differential equations that describe the behavior of the system described in Eq. 36 by standard procedures. Inserting these expressions in Eq. 21, one obtains

$$\begin{aligned}
&G_\Delta^{\alpha,\beta}(t_1, t_2)/[(1/9)(1/3)^{\alpha+\beta}(S_A - S_B)^{\alpha+\beta}] \\
&\quad = 2^\alpha[1 + 2\exp(-3k_{BA}t_1)][1 - \exp(-3k_{BA}t_1)]^\alpha \\
&\qquad \{2^\beta[1 - \exp(-3k_{BA}t_2)]^\beta[2\exp(-3k_{BA}\Delta t) + 1] \\
&\qquad -2[1 + 2\exp(-3k_{BA}t_2)]^\beta[1 - \exp(-3k_{BA}\Delta t)]\} \\
&\qquad -2[1 - \exp(-3k_{BA}t_1)][1 + 2\exp(-3k_{BA}t_1)]^\alpha \\
&\qquad \{2^\beta[1 - \exp(-3k_{BA}t_2)]^\beta[1 - \exp(-3k_{BA}\Delta t)] \\
&\qquad -[1 + 2\exp(-3k_{BA}t_2)]^\beta[\exp(-3k_{BA}\Delta t) + 2]\},
\end{aligned} \tag{37}$$

where $\Delta t = t_2 - t_1$, it being understood that $t_2 > t_1$.

Plots of $G_\Delta^{1,3}(t_1, t_2)$ and of $G_\Delta^{3,1}(t_1, t_2)$ are presented as a function of t_2 in Fig. 5, for the values $(S_A - S_B) = 1$, $k_{BA} = 1\ \mathrm{ms}^{-1}$ and $t_1 = 0.1$ ms. Obviously, the difference between the two correlation functions is very pronounced, which demonstrates that nonstationary noise produced by the system shown in Eq. 36 does not obey time reversal.

DISCUSSION

Even if the system producing a noise signal is stationary, i.e. under steady-state conditions (as is frequently the case experimentally), it may or may not be at thermodynamic

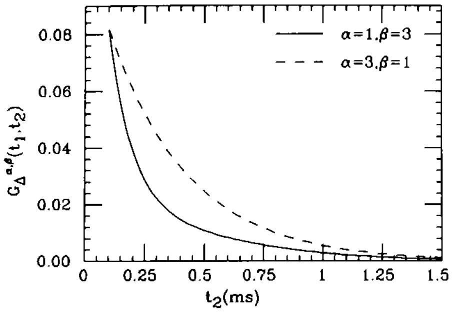

FIGURE 5 A comparison between the autocorrelation functions $G_\Delta^{1,3}(t_1, t_2)$ (solid line) and $G^{3,1}(t_1, t_2)$ (dashed line) of the nonstationary noise produced by a system that is not at steady state. The noise-producing system is the one shown in Eq. 36. At zero time the system starts out in State A. The autocorrelation functions are presented as a function of t_2, t_1 being fixed at a value of 0.1 ms. The values of the parameters used to evaluate the autocorrelation functions presented are: $k_{AB} = 2\ \mathrm{ms}^{-1}$; $k_{BA} = 1\ \mathrm{ms}^{-1}$; $S_A - S_B = 1$.

equilibrium. The noise-generating system may be embedded in an environment that is not at thermodynamic equilibrium, but if it is not coupled to the irreversible processes going on in the environment, it may still have the properties of a system at equilibrium. The crucial point that concerns us here is whether the system responsible for the measured noise obeys or does not obey microscopic reversibility.

We have shown that the noise produced by a system at equilibrium is invariant in its statistical properties to reversal of the arrow of time. If the noise signal is high enough in information (i.e., the various species involved in the noise-producing system have different signal amplitudes in their contribution to the noise) the noise produced by a system that is not at equilibrium will have different statistical properties upon time reversal. Of course, we should use as criteria such statistical properties of the noise that are not inherently invariant to change of the time arrow. We have used generalized forms of the autocorrelation function (see Eq. 3) for this purpose.

In practical cases of the measurement of noise signals there may be contributions from more than one process to the measured fluctuations in the signal. As a specific example, let us consider the case in which there is a background noise where the noise of interest rides on top. Usually, the background noise and the noise signal are not correlated, as we will assume here. Denoting the background noise by $h(t)$, one obtains for $G_\Delta^{1,3}(\tau)$ computed from the measured data of a stationary noise signal,

$$\begin{aligned}
G_\Delta^{1,3}(\tau) &= \langle\{[f(t) + h(t)] - [\overline{f(t) + h(t)}]\} \\
&\quad \cdot \{[f(t+\tau) + h(t+\tau)] - [\overline{f(t) + h(t)}]\}^3\rangle \\
&= \langle\{[f(t) - \overline{f(t)}] + [h(t) - \overline{h(t)}]\} \\
&\quad \cdot \{[f(t+\tau) - \overline{f(t)}] + [h(t+\tau) - \overline{h(t)}]\}^3\rangle \\
&= G_{\Delta,f}^{1,3}(\tau) + 3\,\langle[h(t+\tau) - \overline{h(t)}]\rangle\, G_{\Delta,f}^{1,2} \\
&\quad + 3\langle[h(t+\tau) - \overline{h(t)}]^2\rangle\, G_{\Delta,f}^{1,1} \\
&\quad + \langle[f(t) - \overline{f(t)}]\rangle \cdot \langle[h(t+\tau) - \overline{h(t)}]^3\rangle \\
&\quad + G_{\Delta,h}^{1,3}(\tau) + 3\langle[f(t+\tau) - \overline{f(t)}]\rangle\, G_{\Delta,h}^{1,2} \\
&\quad + 3\langle[f(t+\tau) - \overline{f(t)}]^2\rangle\, G_{\Delta,h}^{1,1} \\
&\quad + \langle[f(t+\tau) - \overline{f(t)}]^3\rangle \cdot \langle[h(t) - \overline{h(t)}]\rangle \\
&= G_{\Delta,f}^{1,3} + 3\langle[\Delta h(t)]^2\rangle\, G_{\Delta,f}^{1,1} + G_{\Delta,h}^{1,3} \\
&\quad + 3\langle[\Delta f(t)]^2\rangle\, G_{\Delta,h}^{1,1}, \qquad (38)
\end{aligned}$$

where $G_{\Delta,f}^{\alpha,\beta}$ and $G_{\Delta,h}^{\alpha,\beta}$ are the autocorrelation functions for the noise of interest and the background noise, respectively. Remembering that $G_\Delta^{1,1}(\tau)$ is always the same for any signal and its time reverse, one may conclude that $G_\Delta^{1,3}(\tau)$ of the noise of interest and of the background noise contribute additively to the time-reversal properties of $G_\Delta^{1,3}(\tau)$. If $G_{\Delta,h}^{1,3}(\tau)$ can be measured independently, or if the background noise is known to obey time reversal, the time-reversal properties of the noise of interest can be conveniently deduced from $G_\Delta^{1,3}(\tau)$. This may be proven true also for $G_\Delta^{1,2}(\tau)$. On the other hand, the relationship between $G_\Delta^{\alpha,\beta}(\tau)$, $G_{\Delta,f}^{\alpha,\beta}(\tau)$ and $G_{\Delta,h}^{\alpha,\beta}(\tau)$ is more complex if $\alpha > 1$ and $\beta \geq 3$ or if $\alpha \geq 1$ and $\beta > 3$, which may provide a good reason against the choice of such values for α and β. Similar considerations apply, of course, to the superposition of any other noise signals that are not mutually correlated.

Another problem that sometimes arises in experiments is a slow drift or run down in the preparation studied. Examples are bleaching processes of the fluorophores in fluorescence experiments or a deterioration in the biological specimen in electrophysiological studies. Note that these processes are, for all practical purposes, slow on the time scale on which the kinetics of the processes of interest take place; otherwise the experiments would be discarded. This being the case, the slow drift does not show up in the time course of $G_\Delta^{\alpha,\beta}(\tau)$, which is evaluated from the data, since the drift is noticeable on time scales τ for which the correlation of interest is vanishingly small anyway. If desired, the slow drift may be picked up by evaluating any function $G_\Delta^{\alpha,\beta}(\tau)$ [including $G_\Delta^{1,1}(\tau)$] for various stretches of the noise trace along the time axis. Drifts in the kinetics behavior of the studied system will be disclosed by changes in $G_\Delta^{\alpha,\beta}(\tau)$ evaluated at different locations of the noise trace.

One can imagine a variety of circumstances when examination of the time-reversal properties of a noise signal may be of interest. As mentioned above, by measurement of the intensity fluctuations of the light emitted upon irradiation of a small number of macromolecules labeled with suitable pairs of donor-acceptor fluorophores, it should be possible to characterize the conformational fluctuations of the macromolecules (Haas and Steinberg, 1984). The time-reversal properties of the fluctuations thus produced by biologically active macromolecules (such as enzymes) may reveal whether the observed conformational transitions are coupled to irreversible metabolic processes. Similarly, by analyzing the fluctuations in the tension of a stretched muscle fiber it might be possible to examine whether these changes in tension are coupled to reversible or irreversible steps in the force-generating process. The motion of macromolecules on cell surfaces may be studied by the method of fluorescence correlation spectroscopy (Elson and Magde, 1974), by which one records the fluctuations in the fluorescence intensity emitted from a small patch of membrane where labeled biopolymers are situated. Analysis of the time reversal properties of the fluorescence fluctuations might indicate whether the motion of the macromolecules in the membrane is brought about by passive diffusion due to thermal motion, or is driven by some irreversible processes. Analysis of the time-reversal properties of noise by the proposed autocorrelation functions can be applied also under certain circumstances to electric noise of excitable membranes to indicate whether metabolic energy is involved in the process of the switch of the ionic channels from one conductance level to another. It is important to note, however, that the autocorrelation functions $G^{\alpha,\beta}(\tau)$ cannot detect thermodynamic nonequilibrium in systems that produce noise that has only two levels of amplitude. Thus, if one of the noise levels is zero, the only contributions to $G^{\alpha,\beta}(\tau)$ are the correlations between stretches of time in which the system exhibits the nonzero level of amplitude. Obviously, for a single nonzero amplitude $G^{\alpha,\beta}(\tau)$ does not change upon time reversal, irrespective of the values of α and β (see Eq. 3). Also, we can show this to be true if both levels of noise (if there are only two such levels) are nonzero. Notwithstanding, there are quite a few cases where the study of the time-reversal properties of ionic conductances by the proposed method is feasible. Some channels do have multiple levels of conductance (Hamill and Sakmann, 1981; Labarca and Miller, 1981; Latorre and Alvarez, 1981). Furthermore, voltage fluctuations under unclamped or current-clamped conditions (Steinberg, 1985) produce multiple level signals even if the conductance has only two levels. Such fluctuations necessarily couple nonequilibrium processes to the opening and closing of channels and are thus expected to violate time reversal. Indeed, this was recently found to be the case when such voltage noise had been evaluated theoretically (Steinberg and Steinberg, to be published). Another case has been presented above, i.e., the nonstationary fluctuations of a two-state system. Strictly speaking, this system exhibits a multilevel rather than a two-level signal, since the measure signal is related to the average signal at any given instant, and the average signal varies from instant to instant. While not amenable to analysis by the approach presented above, two-level single channel recordings permit the examination of the thermodynamic state of the fluctuating ionic channel by a different approach, i.e., by analysis of the statistics of the open and closed durations, as will be shown elsewhere. The present approach, however, has the advantages that it may be applied to multilevel noise, and to noise produced by many entities in the noise-generating system.

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