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Rectification and signal averaging of weak electric fields by biological cells

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ABSTRACT **Oscillating electric fields can be rectified by proteins in cell membranes to give rise to a dc transport of a substance across the membrane or a net conversion of a substrate to a product. This provides a basis for signal averaging and may be important for understanding the effects of weak extremely low frequency (ELF) electric fields on cellular systems. We consider the limits imposed by thermal and "excess" biological noise on the magnitude and exposure duration of such electric field-induced membrane activity. Under certain circumstances, the excess noise leads to an increase in the signal-to-noise ratio in a manner similar to processes labeled "stochastic resonance." Numerical results indicate that it is difficult to reconcile biological effects with low field strengths.**

In previous papers (1, 2) we considered the signal-to-noise ratio expected for a weak external extremely low frequency (ELF) signal field applied to a biological cell subject to thermal (Johnson–Nyquist) noise voltage across the membrane, where the membrane is treated as a resistor and capacitor in parallel. The basic hypothesis is that a biological system cannot in principle be influenced by an applied electromagnetic field if the biological signal (i.e., the change in some parameter away from the unperturbed condition) resulting from the field is smaller than the root-mean-square (rms) noise in that parameter in the absence of the field.

We considered the signal to be the change in the instantaneous membrane potential δV, which leads to the condition $\overline{\delta V^2} \geq 4R_{mem}k_BT\Delta f$ to achieve a signal-to-noise ratio (S/N) greater than unity, where R_{mem} is the membrane resistance, k_B is the Boltzmann constant, T is the absolute temperature, and Δf is the relevant frequency bandwidth.

Since both δV and R_{mem}, as well as the electrical properties that set a limit to Δf, can be written in terms of the size of the cell as well as the membrane electrical properties, estimates for the minimum electric field necessary to at least overcome the effects of thermal noise in terms of the physical parameters of biological cells were obtained.

In the present paper we explicitly treat signal averaging in terms of a general model of field-induced membrane activity that includes the rectification of currents transmitted through the membrane and the rectification of catalytic action by enzymes subjected to fields in the membrane. Also, unlike the previous papers (1, 2), where only the fundamental limit on detection imposed by thermal (k_BT) noise was treated, we consider S/N as a function of a general "white noise" intensity, since there are sources of noise in biological membranes other than Johnson–Nyquist (thermal) noise which may act to effectively increase the temperature. We find that S/N can increase with increasing noise under biologically relevant conditions in a manner similar to the phenomenon of "stochastic resonance" known for many physical systems (3–5).

The Rectifier Equation

Biological cells operate most often near steady state. Molecules that are generated through some process are eliminated at approximately the same rate as they are produced. For example, many metabolic processes (including decarboxylation by ornithine decarboxylase) produce oxidizing radicals, such as superoxide, as by-products. These oxidizing agents can diffuse to the nucleus and oxidize DNA, possibly causing deleterious mutations. The effects of such agents are limited by the action of enzymes such as superoxide dismutase that scavenge these oxidants and convert them to harmless materials. At a steady state, the average rate of production of superoxide equals the rate of degradation, $\overline{J_{creation}} = \overline{J_{destruction}}$, where the overline indicates a time average. However efficient the scavenging mechanism, some radicals will escape the scavengers and be eliminated in the process of oxidizing the genetic material, thus damaging that material. In this picture, DNA acts as a sink for unscavenged radicals, which are turned into damaged sites that accumulate over time.

Through effects on membrane proteins, an applied ELF electric field may increase the average rate of production of superoxide. Specifically, the change in field could affect superoxide production by changing the chemical equilibrium in the cell. That equilibrium could be modified through the field-induced opening of protein gate channels in the membrane, thus changing the membrane transmission properties, or through field-induced changes in the catalytic properties of enzyme proteins associated with the membrane. Such an increase in production will result in an increase in the concentration of the oxidants in the cell and an increased superoxide concentration, leading to an increased damage rate. The biologically important "signal" generated by the field-induced change in membrane activity is the total damage accumulated during exposure to the field.

As is the case for certain models of damage from radioactivity, the important parameter is the accumulated dose and not the maximum instantaneous intensity. To obtain a quantitative relationship between the various relevant parameters (applied field strength, cell size, normal rate of catalysis, noise intensity, and time of exposure) we consider a simple model shown in Fig. 1. Moreover, for clarity, we consider specifically a model for the transmission of a substance S through the membrane where S can enter the cell only through a protein gate or channel. For simplicity, we assume that there are two states of the protein, ψ_{open} and ψ_{closed}. (If the modification follows from a change in the catalytic effect of an enzyme protein imbedded in the membrane, we could label the protein states ψ_{active} and $\psi_{inactive}$ and proceed with the same general argument.)

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Abbreviations: ELF, extremely low frequency; S/N, signal-to-noise ratio.

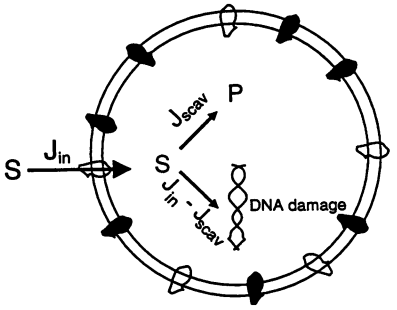

FIG. 1. Schematic illustration of our model. A substance S can enter the cell only through a protein gate. The filled structures indicate closed channels, while the unfilled ones denote open channels through which S can pass. The transition between closed and open state is governed by the membrane potential (see text). After S enters the cell, there are two possible fates—either it is "scavenged" and converted to P or it diffuses to the nucleus and reacts with DNA, causing a damage site. An external electric field will bias the probability for the channel to be open, and hence the current of S into the cell, but the protein responsible for scavenging the S that enters is a cytosolic molecule and not influenced by the field. Thus a relatively small interaction acting over a long period of time can cause accumulation of damaged DNA sites.

The dynamical behavior of the system can be described in terms of diffusion on a one-dimensional potential surface such as depicted in Fig. 2. The equilibrium between the open and closed forms depends on the energy difference between the two states according to a Boltzmann equation $P_{open}/P_{closed} = e^{-U/D}$. The probability for a channel to be open is thus given by the partition equation,

$$P_{open} = \frac{e^{-U/D}}{1 + e^{-U/D}}, \qquad [1]$$

where U is the difference in energy between the closed and open states and D is the amplitude of white noise acting on the channel. At thermal equilibrium, $D = k_BT$ is the characteristic Boltzmann thermal noise energy. Since, for some cells, there may be significant additional stochastic fields of biological origin, we take $D \geq k_BT$, thus approximating the effects of such excess fields as an effective increase in membrane temperature. The number of open channels is $N_{open} = P_{open} \cdot N_{tot}$, where N_{tot} is the total number of channels in the cell membrane.

The average rate of entry of S into the cell, $\overline{J_{in}}$, is proportional to the number of open channels,

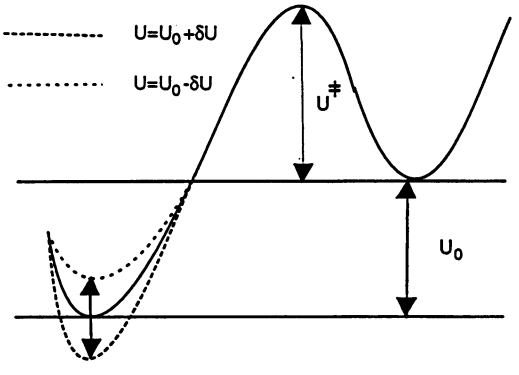

FIG. 2. Illustration of the "free energy" vs. reaction coordinate for the open–closed transition of the membrane channel. When the energy of the closed state is lower than that of the open state, the equilibrium will be shifted toward the closed state. In this case, an applied ac field will cause a net dc increase in the probability for the channel to be open, relative to the unperturbed state.

$$\overline{J_{in}} = k[S]_{out}N_{open}, \qquad [2]$$

where k is a bimolecular rate constant and $[S]_{out}$ is the concentration of S outside of the cell. We take the concentration of S inside the cell to be much less than that outside the cell so the backflow through the open channel can be neglected. The maximum inflow can be defined as $J_{max} = k[S]_{out}N_{tot}$.

We presume a coupling between the gate protein such that an external ELF electric field $E(t) = E_0\cos(\omega t)$ modulates the energy difference between the closed and open states such that $U = U_0 + \delta U$, where U_0 is the energy difference between the states in the absence of a perturbing electric field. At ELF frequencies, where the capacitative admittance of the membrane is very small, $\delta U = 1.5zE_0r_{cell}\cos(\theta)\cos(\omega t)$, where r_{cell} is the radius of the spherical cell, z is the displacement charge of the voltage-gated channel, and θ is the angle between the imposed electric field and the normal to the membrane. We consider $\omega \ll \tau^{-1}$. Here $\tau^{-1} = k_{open} + k_{closed}$ is the inverse relaxation time of the channel, where k_{open} and k_{closed} are the rate constants for opening and closing the channel.

At steady state in the absence of the applied field the average absorption of S in the cell counterbalances the average rate of entry; for $U = U_0$, $\overline{J_{abs}} = \overline{J_{in}}$.

We consider two processes that act to eliminate S, an absorption by scavengers at an average rate, $\overline{J_{scv}}$, and an absorption by an oxidation insult to the DNA with a rate, $\overline{J_{dna}}$, where $J_{dna} + J_{scv} = J_{abs}$. We assume efficient scavenging under equilibrium conditions, so the ratio, $R_0(U = U_0) = J_{dna}/J_{scv} \ll 1$.

In the presence of the applied field, the rate of entry of S into the cell may be increased and the level of S in the cell will increase. But we consider that that increase is small and absorption efficiency will not be changed; hence, $R = R_0$ will be the relevant efficiency parameter for all conditions that we consider.

When the energy U_0 is perturbed by an amount, δU, such that $U = U_0 + \delta U$, J_{in} will be modified and a net current, J, will be generated such that

$$J = J_{in} - J_{in}(0) = J_{max}\left[\frac{e^{-U/D}}{1 + e^{-U/D}} - \frac{e^{-U_0/D}}{e^{-U_0/D}}\right]. \qquad [3]$$

Expanding to second order in $\delta U/D$, for $\delta U \ll D$, we find

$$J \approx J_{max}\frac{e^{-U_0/D}}{(1 + e^{-U_0/D})^2}\left[-\frac{\delta U}{D} + \left(\frac{1}{2} - P_0\right)\left(\frac{\delta U}{D}\right)^2 + \cdots\right]. \qquad [4]$$

Eq. 4 is of the same general form as that describing the properties of a diode rectifier (6). The effect of an oscillating field is always to bring the average ratio between the open and closed states of the channel closer to unity. Thus, if $P_0 < \frac{1}{2}$ (where $P_0 \equiv P(U = U_0)$), the number of open channels, and hence the average rate of entry of S into the cell increases due to the applied field.

Averaging over time and θ gives the change in the average rate of accumulation over the surface of a spherical cell (7);

$$\bar{J} \approx \frac{e^{-U_0/D}}{D^2}AJ_{max}(zE_{rms}r_{cell})^2, \qquad [5]$$

where we use $E^2_{rms} = E_2^0/2$ for simpler comparison of the results with the conventional description of measured fields and we have defined

$$A = \frac{3(\frac{1}{2} - P_0)}{32(1 + e^{-U_0/D})^2},$$

which is bounded between -0.002 and 0.05.

When U_0 is zero, the system is symmetric, and P_0 the second-order term in Eq. **4**, as well as $\bar{J}$, are identically zero. When U_0 is not zero, the imposition of the ELF electric field generates a rectified excess flow of S across the membrane, $\bar{J}$. That increased flow will result in a commensurate increase in the absorption, and a portion R of those absorption events will result in injury to DNA. In a time t we expect an accumulation of $(\bar{J}t \cdot R)$ injury events due to the applied field. Because both the inflow and the scavenging processes are stochastic, there will also be a "noise" flow which is the variance of the sum of the two processes. If we express the flow in molecules per second, the net noise drift across the membrane is equal to the square root of the number of molecules passing through the membrane plus the number absorbed. At steady state—with no external field—the probable drift in a time t is

$$Q_{\text{noise}} = (2Rk[\text{S}]_{\text{out}}N_{\text{open}}t)^{1/2} \quad [6]$$

molecules. This "noise" is to be compared with the signal—i.e., the molecular transport induced by the ELF field in a time t; $Q_{\text{sig}} = \bar{J}t$. After straightforward algebra we find

$$\text{S/N} \approx \frac{e^{-U_0/2D}}{D^2} A'(J_{\max}Rt)^{1/2}(zE_{\text{rms}}r_{\text{cell}})^2, \quad [7]$$

where $A' = (0.5A^2)^{1/2}(1 + e^{-U_0/D})^{1/2}$, which is bounded between 0 and 0.025.

This equation is of the same general form as that given by McNamara and Wiesenfeld in their theory of stochastic resonance (5), but with a time dependence indicative of the second-order rectification process on which we focus in this paper. This is perhaps not surprising, since the fundamental picture motivating our model is a double-well potential (Fig. 2) similar to that considered by McNamara and Wiesenfeld. In our case, however, the potential is not necessarily symmetric (i.e., the energy at the bottom of one well is different than at the bottom of the other well).

In Fig. 3 we show a plot of S/N vs. D, for a value of the energy gap, $U_0 = 8k_BT$, which shows the increase in S/N with noise, at small values of noise, characteristic of stochastic resonance. When the two canonical states are separated by an energy, U_0, greater than the energy of the applied field, $zE_{\text{rms}}r_{\text{cell}}$, that field cannot bridge the energy gap and induce a transition in the absence of noise. For noise amplitudes less than about $1/e$ of the gap, the value of S/N will increase with the noise, for larger values of the noise, S/N will fall off with increasing noise.

To obtain an estimate for the combination of time of exposure and applied field amplitude necessary to achieve a S/N of unity, we solve Eq. **7** for the applied field amplitude $E_{\text{o,min}}$, such that the field-altered net transport will equal the probable uncertainty in the transfer through noise in a time t to find

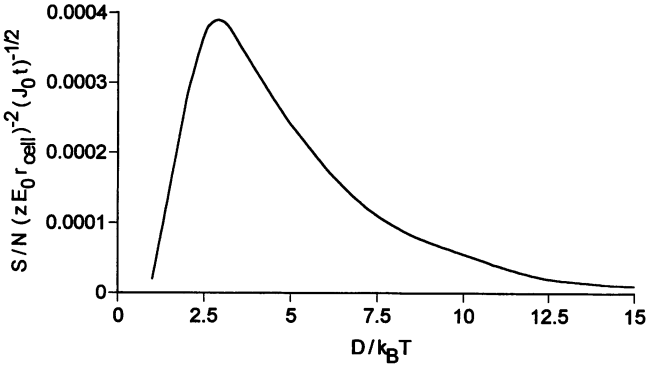

FIG. 3. Plot of S/N vs. the noise intensity, D, calculated from Eq. **7** with $U_0 = 8k_BT$.

$$E_{\min} \approx \frac{De^{U_0/4D}}{zr_{\text{cell}}}(A'RJ_{\max}t)^{-1/4}. \quad [8]$$

Homeostatic Constraints. Eqs. **3–8** take the form presented in the absence of homeostatic constraints. In some circumstances, cell physiology will dictate a steady-state value of Q in the cell, Q_{equil}, such that, to first order in $\Delta Q = Q - Q_{\text{homeostasis}}$,

$$J_{\text{homeostasis}} = \frac{dQ}{dt} = -\frac{1}{\tau_{\text{homeostasis}}}(Q - Q_{\text{equil}}), \quad [9]$$

where $\tau_{\text{homeostasis}}$ is a time constant for the return to steady state of a perturbed cell. In such a case, a term like that on the left of Eq. **9** should be added to Eq. **3** for a complete description of the change in S (or Q) in the cell.

In the continuous presence of the perturbation, a new steady state will be defined. If that steady-state situation is such as to generate a higher level of stochastic insult to the cell—e.g., as injury to DNA—the probability of additional insults will be proportional to the time under which the perturbation is imposed and no pertinent homeostasis may be relevant. We have proceeded in the conservative approximation that homeostatic effects are not important.

Numerical Results

There are four parameters to consider. These are the product of the applied field E and the coupling factor zr_{cell}; the product of a maximum background flow $J_{\max}$ and time t; the unperturbed energy difference between open and closed states U_0; and the "white" noise intensity D. As an explicit example, consider a 100-μm cell exposed to a 1-mV/cm rms field in the aqueous medium surrounding the cell.

If we take $U_0 = 8k_BT$, $J_{\max} = 10^{12}$/sec and $z = 10$, we find that S/N = 1 is reached after 10,700 sec (= 3 hr) for $D = k_BT$. If instead, $D = 4k_BT$ ($D = U_0/2$ minimizes $E_{\min}$ in Eq. **8**), we find that S/N = 1 is reached after 3000 sec (1 hr).

The minimum field necessary to cause an effect in a reasonable period of time is greater than predicted previously. This is principally due to the fact that in ref. 1 the field itself was compared to the thermal Johnson–Nyquist noise field, and the signal averaging was incorporated into that picture. Here, we have looked at a specific mechanism involving interaction between an applied field and a membrane protein more realistically.

The change in the average behavior of the protein depends on the square of the applied field. Also, since we adopt the viewpoint that the effect of the field is to change the rate of entry or production of some molecule, we compare our "signal" with the shot noise arising from the discreteness of the molecular events. The number of such events at the membrane cannot possibly be greater than the total diffusive flux of the substrate to the membrane surface. For reasonable parameters, this flux is about 10^{12} events per second at most, so the shot noise is about $\pm 10^6$/sec. Although the limits imposed by the present theory are higher than those of Weaver and Astumian, they are still within an order of magnitude of the experimental data used as benchmarks in ref. 1.

Other experiments, principally those using magnetic fields, have reported effects at very low field intensities, however. For example, a 5-mG 60-Hz field (such as implicated in epidemiological studies) produces an electric field of 0.23 μV/cm at the widest girth of a human form. This may seem very small, but from the structure of the equations we see that we only have to wait in order for the field to be rectified and produce a signal larger than noise. How long must we wait? From Eq. **7**, we find that a 0.23-μV/cm field will give rise to a S/N of unity only after $4 \cdot 10^{18}$ sec, which is a long time. Thus, it seems

difficult to reconcile effects with low field strengths in the context of the present theory.

Conclusion

We have discussed two mechanisms that may be relevant for understanding possible effects of weak electric fields on biological cell-rectification by enzymes and transporters, which allows a dc response to be generated by an ac signal, and stochastic resonance, which allows "white" noise acting on a system to give rise to a larger signal (and S/N) than would be the case if only thermal (k_BT) noise were acting on the system. We focused on a specific model of field-induced membrane activity where a substance flows through a membrane voltage-gated channel, but the basic principles apply to a much wider array of physical and chemical systems (8). The nonmonotonic dependence of S/N on noise strength can be expected in any system that must surmount an activation barrier to make a transition resulting in a response to an external signal. Rectification requires in addition an asymmetry. In the case of the channel, the asymmetry is provided by $U_0 \neq 0$.

In conclusion, the two major results of this paper are (*i*) that rectification provides a mechanism by which signal from an external ac electric field can be accumulated; and (*ii*) that "noise" larger in magnitude than expected at equilibrium does not necessarily lead to a higher threshold for response of a system to a weak ELF field, and indeed can increase the ability of the system to respond. Despite these conclusions, we must remember that the field strengths predicted as thresholds for response, while small, are still larger than fields likely to arise from typical environmental sources.

Further, it is important to emphasize that even if an external field is larger than our threshold value, this does not imply that the field will cause an effect, but only that an effect is possible within the context of a straightforward thermodynamic perturbation–response analysis.

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