Theoretical Limits on the Threshold for the Response of Long Cells to Weak Extremely Low Frequency Electric Fields Due to Ionic and Molecular Flux Rectification

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ABSTRACT Understanding exposure thresholds for the response of biological systems to extremely low frequency (ELF) electric and magnetic fields is a fundamental problem of long-standing interest. We consider a two-state model for voltage-gated channels in the membrane of an isolated elongated cell ($L_{cell} = 1$ mm; $r_{cell} = 25$ μm) and use a previously described process of ionic and molecular flux rectification to set lower bounds for a threshold exposure. A key assumption is that it is the ability of weak physical fields to alter biochemistry that is limiting, not the ability of a small number of molecules to alter biological systems. Moreover, molecular shot noise, not thermal voltage noise, is the basis of threshold estimates. Models with and without stochastic resonance are used, with a long exposure time, $t_{exp} = 10^4$ s. We also determined the dependence of the threshold on the basal transport rate. By considering both spherical and elongated cells, we find that the lowest bound for the threshold is $E_{min} \approx 9 \times 10^{-3}$ V m^{-1} (9×10^{-5} V cm^{-1}). Using a conservative value for the loop radius $r_{loop} = 0.3$ m for induced current, the corresponding lower bound in the human body for a magnetic field exposure is $B_{min} \approx 6 \times 10^{-4}$ T (6 G). Unless large, organized, and electrically amplifying multicellular systems such as the ampullae of Lorenzini of elasmobranch fish are involved, these results strongly suggest that the biophysical mechanism of voltage-gated macromolecules in the membranes of cells can be ruled out as a basis of possible effects of weak ELF electric and magnetic fields in humans.

INTRODUCTION

Long-standing interest in the effects of weak, extremely low frequency (ELF) electric and magnetic fields on biological systems has arisen from two questions. First, what are the biophysical mechanisms that underlie the navigational response of some animals to weak electric fields in the ocean (Kalmijn, 1982) or small differences in the earth's magnetic field (Wiltschko and Wiltschko, 1996)? Second, what can be understood about the possible basis of reported biological effects associated with 50- to 60-Hz fields that accompany the distribution and use of electric power (National Academy of Sciences, 1997)? Our approach conceptually separates the problem into two parts. First, the physical fields have to influence some biochemical process. Second, this biochemical change must influence the larger biological system.

The second step poses no problem because exposure of a biological system to a small number of molecules clearly can alter that system. Evolved sensory systems (olfaction and taste) can respond to a small number of molecules, and hormones at low levels cause significant effects. Likewise, introduction of only 400 molecules of bleomycin into an isolated mammalian cell results in apoptosis (Mir et al., 1988).

In contrast, there are significant limits on the first step, the ability of weak fields to alter biochemistry through known biophysical mechanisms. A crucial concept that has been introduced previously is that of a "potentially causal molecular change" (Astumian et al., 1995). Namely, if the physical field is to have a downstream effect on the biological system, it must perturb a biochemical process enough that the perturbation is noticeable amidst the usual biological variability. Note that this is only a necessary condition for a downstream effect, not a sufficient one, which is why the molecular change caused by the field is regarded as only potentially causal. Thus, the challenge is to achieve quantitative understanding of how exposure to these weak physical fields could result in potentially causal molecular changes.

METHODS

Previous physical theory methods

Most estimates of weak field thresholds have been based on physical analyses in which the field-induced change in a physical quantity (e.g., the cellular transmembrane voltage) is compared to fluctuations in the same physical quantity (e.g., thermal noise) (Weaver, 1987; Weaver and Astumian, 1990; Adair, 1991; Weaver and Astumian, 1992; Polk, 1992). Such approaches are technically correct but can be incomplete and therefore misleading. Without explicitly including the coupling of the physical field to a biochemical process, the impact of an exposure on biochemistry is unknown. Also, it is widely accepted that weak ELF fields implicated by epidemiology have molecular interaction energies insufficient to break chemical bonds (Valberg et al., 1997). Thus the only possible effect of weak fields would be to alter existing transport and reaction rates.

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Molecular shot noise yields a fundamental limit

When attempting to distinguish an altered rate (due to field exposure) from an unaltered one, a fundamental threshold arises because of the stochastic nature of essentially all biochemical processes beyond DNA replication. Specifically, at the completion of either a control exposure (zero field) or a test exposure (nonzero field), the net molecular change exhibits statistical fluctuations. These are fundamental because the microscopic events underlying molecular transport (e.g., diffusion) and chemical reactions (e.g., enzyme catalysis) involve large numbers of randomizing collisions or conformational variations. For this reason, Poisson statistics governs the distribution of end point molecular changes (or cumulative flux through a biochemical pathway) around a mean $\bar{n}$, with standard deviation (uncertainty) $\sigma = \sqrt{\bar{n}}$. Because $\bar{n}$ is large, the Poisson distribution is very well approximated by a Gaussian distribution with the same mean and standard deviation. This uncertainty is "molecular shot noise." This provides a basis for using a signal-to-noise ratio criterion, $(S/N) \approx 1$, to estimate exposure thresholds.

It is important to recognize that this limit is not based on thermal noise in the membrane voltage, as in earlier estimates (Weaver, 1987; Weaver and Astumian, 1990; Adair, 1991; Weaver and Astumian, 1992). Instead, the threshold criterion is obtained from fundamental stochastic variability in the end point of a biochemical process, here voltage-gated mediated transport. Stochastic fluctuations arise from many biochemical processes, such as aqueous diffusion and protein "breathing motions," in addition to transmembrane voltage noise. Thus, molecular changes within biochemical pathways are thoroughly randomized, with average values controlled but containing an inherent molecular shot noise uncertainty.

General features of alteration of biochemical rates by periodic electric fields

The first application of this approach (Astumian et al., 1995; Astumian et al., 1997) treated a large, isolated spherical cell and utilized a two-state model that represents an entire class of biophysical mechanism, voltage-gated cell membrane proteins (channels and enzymes). In this case a slight alteration of the ion or molecular transport through voltage-gated channels was treated.

In the case of voltage-gated proteins, as an illustrative example, we look for a potentially causal molecular change in the number of Ca^{2+} ions that enter the cell via the channels. Note that we are trying to show *quantitatively* that we can distinguish a cell that is exposed to an electric field from one that is not.

The net molecular change (number of ions entering the cell) following an exposure is

$$\bar{n} = \bar{n}_0 + \bar{n}_S, \tag{1}$$

where $\bar{n}$ is the average molecular change, $\bar{n}_0$ is the average (basal) change for zero field exposure, and $\bar{n}_S$ is the additional average change due to the field exposure. We regard the field-induced molecular change as a "signal" $S = \bar{n}_S$, so that it can be compared with competing molecular changes from other sources.

For periodic (ac) electric fields the molecular change signal is

$$S = \bar{n}_S = K_{bpm,ac} E^2 J_{max} t_{exp}, \tag{2}$$

where $K_{bpm,ac}$ describes the coupling to an ongoing biochemical process by the periodic electric field for a particular biophysical mechanism; and J_{max} is the maximum possible influx rate, which occurs when all the channels are open.

Choice of elongated cell

A previous analysis considered a large ($r_{cell} = 100\ \mu m$) isolated spherical cell and the maximum cumulative molecular change due to voltage-gated membrane proteins (Astumian et al., 1995). A "most-field-sensitive" version of the theoretical model was used by choosing model parameters that exaggerated the molecular change. This approach is conservative and yields a lower bound for the threshold. This included use of a very large gating charge and basal rate. For a typical exposure $t_{exp} = 10^4$ s, the threshold magnitude of the extracellular electric field was found to be $E_{min} \approx 10^{-3}$ V cm^{-1}.

Here we consider the possibility that still larger cells, in the form of very elongated cells, might lead to a lower minimum threshold. This could occur because the same field creates progressively larger transmembrane voltage changes as the cell size increases, until the cell size reaches the space constant, λ_{cell} (Gaylor et al., 1988). For a most-field-sensitive case, we used a long cylindrical cell with flat end caps (Fig. 1; $L_{cell} = 1000\ \mu m = 1$ mm; $r_{cell} = 25\ \mu m$).

Voltage-gated channel-mediated Ca^{2+} influx

For consistency, here we scaled $J_{max} = 10^{12}$ s^{-1} used in the spherical cell case (Astumian et al., 1995), by using the same maximum flux per membrane area. Specifically, we used the scaling factor $F_{scale} = 2\,A_{end\ cap}/A_{spherical\ cell}$, where $A_{end\ cap} = \pi r^2_{end\ cap}$ and $A_{spherical\ cell} = 4\pi r^2_{spherical\ cell}$, which gives $F_{scale} = 5 \times 10^{-3}$. Thus, we used a maximum possible Ca^{2+} influx (through both long cell end caps) of $J_{max} = 5 \times 10^9$ s^{-1}.

Note that we considered the case in which Ca^{2+} influx occurs only across the ends of the cell, which corresponds to assuming that all of the voltage-gated calcium channels are located within these regions of the cell membrane. This choice was made to exaggerate alteration of the Ca^{2+} influx by the electric field, because in this case there is no field-insensitive influx, which if present would contribute additional stochastic fluctuations but no field-altered changes. For the long cell treated here, $K_{bpm,ac}$ is

$$K_{bpm,ac} = \frac{e^{U_0/D}}{4D^2} P_0^2 \left(\frac{1}{2} - P_0\right)(zL_{cell})^2, \tag{3}$$

where U_0 is the barrier at the resting transmembrane potential, D is the noise energy density, $P_0 = e^{-U_0/D}/(1 + e^{-U_0/D})$ is the probability that a channel is open, z is the channel gating charge (in multiples of the electronic charge, $e = 1.6 \times 10^{-19}$ C), and L_{cell} is the length of the cell. For a conservative most-field-sensitive model we set z to a typical large value; here we use $z = 10e$ (Astumian et al., 1995).

Following the general approach used previously (Astumian et al., 1995), we determine that the threshold extracellular electric ELF field for the elongated cell is

$$E_{min,ELF} \approx \frac{2De^{-U_0/2D}}{zL_{cell}} P_0^{-1}\left(\frac{1}{2} - P_0\right)^{-1/2} (J_{max} t_{exp})^{-1/4} \tag{4}$$

for exposure time $t_{exp} = 10^4$ s.

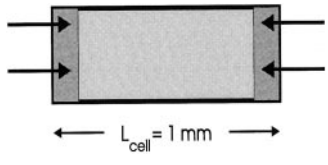

FIGURE 1 Drawing of the isolated large elongated cell that is intended to exaggerate the response of any actual cell. To achieve a conservative most-field-sensitive version of the model it is assumed that the Ca^{2+} influx is confined to the end caps of the cell (*arrows*). This assumption eliminates contributions of stochastic fluctuations in a field-independent Ca^{2+} flux across the side walls of the cell. To further exaggerate the molecular or ionic change due to the field the full cell length $L_{cell} = 1$ mm is used (neglecting the space constant) and a representative value of $r_{cell} = 25\ \mu m$ is used for the cell radius.

We first considered the case of thermal noise ($D = kT$) and a typical barrier ($U_0 = 8\ kT$). This yields a threshold value $E_{min} \approx 0.1$ V m^{-1} ($E_{min} \approx 10^{-3}$ V cm^{-1}). We also considered the optimal stochastic resonance conditions by using excess noise of $D = 4\ kT$. This yielded a slightly smaller threshold, $E_{min} \approx 4 \times 10^{-2}$ V m^{-1} ($E_{min} \approx 4 \times 10^{-4}$ V cm^{-1}). Note that the large spherical cell, which has a larger area of participating membrane, has a lower predicted threshold for the $D = 1\ kT$ case, and a comparable predicted threshold for the $D = 4\ kT$ case.

Dependence of threshold on basal rate

In our model there are a fixed number of channels. The operating point of the cell for a calcium influx is therefore determined by its extracellular concentration, [Ca^{2+}], the number of Ca^{2+} channels, and the probability

$$P = \frac{e^{-U/D}}{1 + e^{-U/D}} \tag{5}$$

of a channel being in the open (conducting) state. The barrier

$$U = U_0 + \Delta U = U_0 + z\Delta V_m \tag{6}$$

can be altered by changing the transmembrane voltage, and this determines P_0, and therefore the mean (basal) rate under resting conditions (Fig. 2). The electric field threshold can be lowered somewhat by shifting the operating point to the optimal point for weak field detection. For the $D = 1\ kT$ case, the threshold can be lowered by an order of magnitude (Fig. 3). For the $D = 4\ kT$ case, the threshold is lowered only slightly (Fig. 4).

Human magnetic field threshold

The corresponding magnetic field thresholds for humans are estimated by considering the magnetic field needed to induce the threshold electric field. For simplicity, we consider a circular loop within the tissue of the human body. Conservatively, we use a loop radius $r_{loop} = 0.3$ m, so that the magnetic field exposure has a magnitude

$$B_{min} \approx \frac{E_{min}}{\pi r_{loop} f}, \tag{7}$$

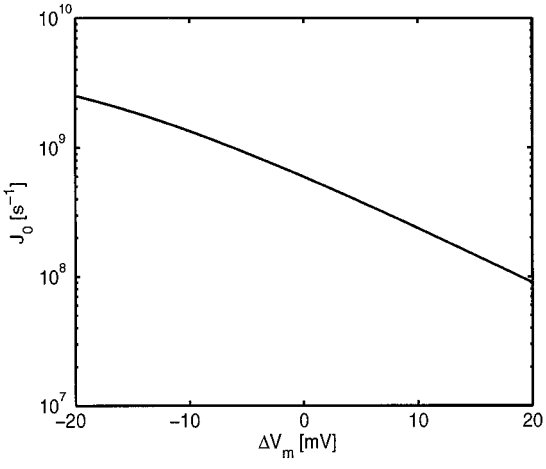

FIGURE 2 Semilog plot of J_{max}, the exaggerated basal Ca^{2+} influx for the simple two-state channel model, in which a biasing voltage (ΔV_m) is varied to alter the average fraction of open channels. Here we used a barrier $U = U_0 + z\Delta V_m$ with $U_0 = 8\ kT$ and a large gating charge, $z = 10e = 1.6 \times 10^{-18}$ C.

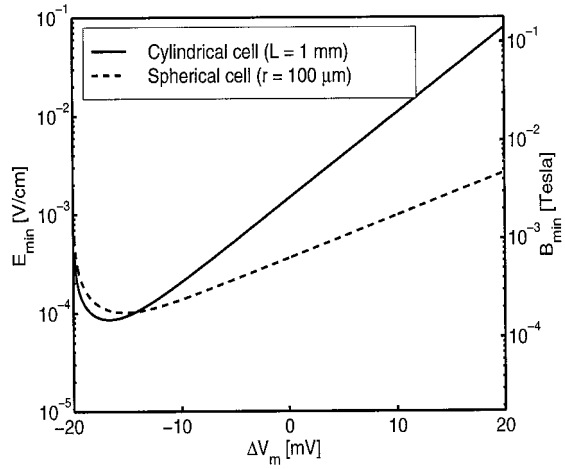

FIGURE 3 Semilog plot of the electric and magnetic field magnitudes for threshold exposures using an exposure time $t_{exp} = 10^{-4}$ s. The dotted line is the result for the spherical cell model (Astumian et al., 1995); the solid line for the long cell model. As expected from the $J_{max}^{-1/4}$ dependence of Eq. 4 the threshold increases significantly as the basal rate is diminished (here, by considering larger values of ΔV_m). As progressively smaller values of (ΔV_m) are used, the influx rate increases and initially the threshold decreases, reaching a minimum value of $9 \times 10^{-4}\ V\,cm^{-1}$ for the large spherical cell, and $3 \times 10^{-3}\ V\ cm^{-1}$ for the large elongated cell. As the basal rate becomes very large for very negative values of ΔV_m the threshold again rises, because the electric field-induced transmembrane voltage change creates only a small fractional change in the rate.

where $f = 50$ or 60 Hz is the ELF frequency. For $t_{exp} \approx 10^4$ s the value is $B_{min} \approx 3 \times 10^{-3}$ T (30 G) for $U_0 \approx 8\ kT$ and $D = kT$. This can be lowered to $B_{min} \approx 10^{-4}$ T (1 G) by shifting the transmembrane voltage as discussed above (Fig. 3). If optimal stochastic resonance is included in this otherwise most-field-sensitive model, the corresponding value becomes $B_{min} \approx 7 \times 10^{-4}$ T (7 G) for $U_0 \approx 8\ kT$ and the excess noise density $D = 4\ kT$, and can be lowered only to $B_{min} \approx 6 \times 10^{-4}$ T (6 G) by shifting the transmembrane voltage (Fig. 4).

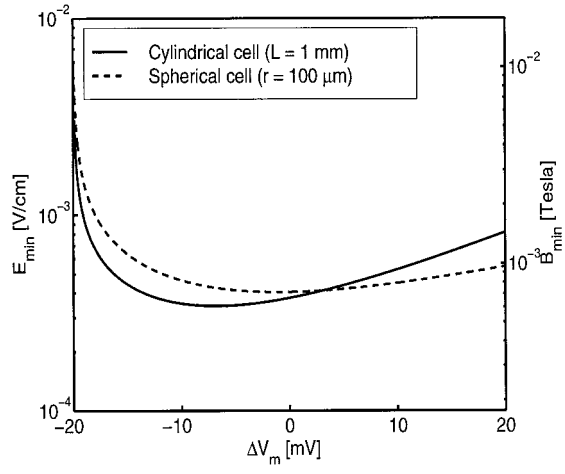

FIGURE 4 Semilog plot as in Fig. 3 but for the case of a larger ("excess") noise density $D = 4\ kT$ to obtain optimal stochastic resonance, and a barrier magnitude $U_0 = 8\ kT$ at the cells' resting transmembrane voltage ($\Delta V_m = 0$). In this case, the spherical cell always has a lower threshold than the $D = 1kT$ threshold. The minimum threshold electric field magnitude is $E_{min} \approx 4 \times 10^{-2}$ V m^{-1} ($4 \times 10^{-4}\ V\ cm^{-1}$), and the smallest threshold magnetic field for the human body is about $B_{min} \approx 4 \times 10^{-4}$ T (4 G).

RESULTS AND DISCUSSION

We use a "most-field-sensitive" version of the model that leads to lower bounds for the threshold exposure that creates a potentially causal molecular change. Specifically, we use a large value of the channel's displacement charge ($q = 10e$), a large basal flux (10^8 to 3×10^9 s^{-1}) which is varied by allowing the resting transmembrane voltage to take on different values ($\Delta V_m = -20$ to $+20$ mV; Fig. 2), optimal stochastic resonance (noise density $D = 4\ kT$; Fig. 4), and a cell length ($L_{cell} = 1$ mm) that exceeds usual space constant values. Nevertheless, the elongated cell often has higher thresholds than the spherical with $r_{cell} = 100\ \mu$m, owing to the smaller cell membrane area.

The lower bounds to a fundamental threshold are not small values, and depend significantly on the barrier U. For small barriers and correspondingly large basal rates the biochemical process is almost "fully turned on." This means that the opportunity for regulatory control, a hallmark of biological systems, becomes lost as J_{max} is increased. That is, biological systems contain many different molecules that have the potential to react but spontaneous rates are generally low, so that highly specific biochemical processes such as enzyme catalysis and receptor binding can provide control and regulation of specific biochemical pathways. Our analysis shows that an attempt to achieve smaller response thresholds by decreasing the barrier will cause the system to become progressively less biological. Conversely, biological systems with specific control have significant barriers (e.g., $U_0 \approx 8\ kT$), and this leads to higher thresholds (Figs. 3 and 4).

Moreover, invoking the longest plausible cell subject to the limitations of the space constant does not provide an explanation for the existence of molecular changes due to the weak fields (mG range) implicated by epidemiology. Unless an appropriately organized multicellular structure is present, as in the ampullae of Lorenzini of the elasmobranch fish (Kalmijn, 1982), it appears that the ubiquitous biophysical mechanism of voltage-gated cell membrane proteins cannot be a basis for possible biological effects in humans due to weak (mG level) 50- to 60-Hz magnetic fields.

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