Biological Effects Due to Weak Electric and Magnetic Fields: The Temperature Variation Threshold

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ABSTRACT

A large number of epidemiological and experimental studies suggest that prolonged (>100 s) weak 50-60-Hz electric and magnetic field (EMF) exposures may cause biological effects (NIEHS Working Group, NIH, 1998 ; Bersani, 1999). We show, however, that for typical temperature sensitivities of biochemical processes, realistic temperature variations during long exposures raise the threshold exposure by two to three orders of magnitude over a fundamental value, independent of the biophysical coupling mechanism. Temperature variations have been omitted in previous theoretical analyses of possible weak field effects, particularly stochastic resonance (Bezrukov and Vodyanoy 1997a . *Nature.* 385:319-321; Astumian et al., 1997 *Nature.* 338:632-633; Bezrukov and Vodyanoy, 1997b . *Nature.* 338:663; Dykman and McClintock, 1998 . *Nature.* 391:344; McClintock, 1998; Gammaitoni et al., 1998 . *Rev. Mod. Phys.* 70:223-287). Although sensory systems usually respond to much shorter (~1 s) exposures and can approach fundamental limits (Bialek, 1987 *Annu. Rev. Biophys. Biophys. Chem.* 16:455-468; Adair et al., 1998 . *Chaos.* 8:576-587), our results significantly decrease the plausibility of effects for nonsensory biological systems due to prolonged, weak-field exposures.

INTRODUCTION

Weak fields are incapable of directly breaking chemical bonds (Valberg et al., 1997). Thus, if weak fields are the basis of biological effects, these can only occur by the alteration of ongoing biochemical reaction or transport processes (Astumian et al., 1995 ; Weaver et al., 1998). But the rates of these processes depend significantly on temperature. To illustrate consequences of realistic temperature variations, we consider a single process with rate in units of molecules per time. $J(T)$ has a weak temperature dependence through the frequency factor (T) and a strong dependence through the Boltzmann factor, $\exp[-U_0/kT]$, where U_0 is an activation energy barrier and kT is the thermal energy. $J(T)$ also depends on the concentrations of ionic or molecular species, which here are assumed to be constant to focus on temperature variations. This relatively simple expression provides a reasonable quantitative description of the temperature dependence of many biochemical reactions, voltage-gated cell membrane channel transport, and molecular and ionic diffusion within aqueous media. Such processes are ubiquitous in biological systems, so it would be difficult for such systems to escape the temperature dependence of these processes.

A more general formulation uses stochastic resonance with a noise density $D \quad kT$ (Astumian et al., 1995). The temperature sensitivity is often described by a first-order coefficient, $_T = (1/J)(\ J/\ T)$, with $_T = U_0/kT^2$ for Eq. 1, but using only the Bolzmann factor. Typically $U_0 \quad 8kT$, so $_T \quad 0.03°C^{-1}$. The net temperature coefficient of more complex, multiple rate processes can be measured; in some cases it is very small or even negative, because of entropic effects, but it is seldom zero.

Biological systems experience significant temperature variations. Human core body temperature undergoes daily variations greater than 1°C (Hammel, 1968 ; Rubin, 1987 ; Keatinge et al., 1986 ; Shiraki et al., 1988 ; Webb, 1992), with larger variations in the extremities. In vitro electric and magnetic field experiments use feedback control (e.g., temperature-regulated exposure chambers), with variations greater than ~0.01°C for exposure times of more than ~100 s (Appendix A). Significantly smaller in vitro variations (e.g., ±0.002°C) are achieved only with nontrivial effort (Fulton et al., 1980).

Temperature variations cause molecular changes by varying J, which together with fundamental stochastic fluctuations compete with the molecular change due to the field exposure. This competition defines a lower bound to a response threshold. For zero field and steady temperature, an ongoing biochemical process is presumed to proceed at a quasisteady (basal) rate J_0, with a molecular change after an exposure time t_{exp} of $_0$ $J_0 t_{exp}$. This is the average number of molecules passing through a biochemical pathway or accumulating at an end point.

A weak field alters J slightly, via a biophysical mechanism such as voltage-gated cell membrane proteins (Astumian et al., 1995 ; Weaver et al., 1998), creating an additional field-induced molecular change:

S denotes the molecular change "signal" that arises from a periodic (ac) electric or magnetic field $F = F_0 \cos \quad t$, and $K_{bpm,ac}$ describes the coupling of the ac field to the biochemical process. The total molecular change is the basal change $_0$ plus the slight additional change $_S$:

To determine whether $_S$ could be the possible basis of a biological effect, competing molecular changes in the same biochemical pathway are considered. "Molecular shot noise" is a fundamental and inescapable source of fluctuations (Astumian et al., 1995 ; Weaver et al., 1998). Essentially all biochemical processes other than DNA replication are stochastic, because of thermal fluctuations and excess noise within biochemical and cellular systems. The throughput or end point change, , is therefore described by Poisson statistics, with an inherent uncertainty (noise) $N =$ (Villars and Benedek, 1974). This leads to a fundamental signal-to-noise ratio criterion that determines necessary, but not sufficient, conditions for a biological effect. The condition $S/N \quad 1$ provides the minimum (threshold) field magnitude,

Each class of biophysical mechanism (e.g., voltage-gated membrane proteins, magnetically sensitive radical pair reactions, magnetite-mediated mechanisms, electrocompression of extracellular matrix) will have a different strength and mathematical expression for the ac coupling, $K_{bpm,ac}$, of the field to J. The critical issue is whether the molecular change in the exposed system, n_{exp}, can be distinguished from a control (nonexposed) system change, n_{con}.

Changes in both systems have stochastic variations approximately equal to , but they differ by $_S$, the molecular change due to the field exposure. Thus

Temperature variations create additional uncertainty. Systematic temperature differences could lead to false positives, in which responses are mistakenly attributed to weak fields. For example, $T \quad 10^{-5}$°C with $_T = 0.03°C^{-1}$ creates the same molecular change as a fundamental threshold exposure (Weaver et al., 1998) for voltage-gated channels in a long cell. Conservatively, we assume that any systematic temperature difference between the exposed and the control systems has been eliminated. Instead, the temperature regulation parameters are assumed to be randomly distributed about the same mean value for both

systems. The molecular change variations due to temperature regulation variability are defined as $n_V = V$. Equation 5 is therefore expanded to
We adopt an expanded signal-to-noise criterion,
with independent uncertainties adding in quadrature. Equations 2 and 7 yield the temperature variation threshold, illustrated here by including both steady (dc) temperature offsets and periodic (ac) temperature variations shown in Fig. 1. Significantly, the temperature variation threshold is much larger than the fundamental threshold (Eq. 4). Fig. 2 shows R_{thresh}, the ratio of the temperature variation threshold field magnitude (Eq. 7) to the fundamental threshold (Eq. 4). For a typical barrier $U_0 = 8kT$, $R_{thresh} = 150\text{-}500$ if $T_{dc} = 0.01\text{-}0.1°C$ (typical in vitro minimum variation), and $R_{thresh} = 1600\text{-}2800$ for $T_{dc} = 1\text{-}3°C$ (human in vivo minimum variation) for long exposures.

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FIGURE 1 The most important parameters in the temperature history of the exposed and control biological systems are the time-averaged temperature, $T_{dc,exp}$ and $T_{dc,con}$, followed by the magnitude of a sinusoidal temperature variation representing temperature regulation, $T_{ac,exp}$ and $T_{ac,con}$, respectively. The complete parameterization is
where the terms in brackets indicate quantities with random errors of the magnitude indicated. Our estimates are conservative in the sense that the systematic temperature difference between the systems is zero ($T_{dc,exp} = T_{dc,con}$). We also neglect differences in the sinusoid periods, phases, and other parameters (e.g., slight differences in exposure time), whose random distribution would further increase temperature variability (details in Appendix B).

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FIGURE 2 Dependence of R_{thresh}, the ratio of the temperature variation threshold to the fundamental molecular shot noise threshold, on barrier height, for a biochemical process described by Eq. 1. The dark-shaded band corresponds to in vivo temperature variations, with T_{dc} = 1-3°C; the light-shaded band corresponds to in vitro temperature variations, with T_{dc} = 0.01-0.1°C. This result is independent of the biophysical mechanism (voltage-gated channels, radical pair reactions, magnetite-medicated processes, etc.) involved in coupling a weak 50-60-Hz field to an ongoing biochemical process. Typical processes have U_0 8kT. Those with large U_0 have high fundamental thresholds, whereas those with small U_0 depart from "biological conditions" in that the spontaneous rates are large and, in the absence of a rate-limiting barrier, lack biological control (Weaver et al., 1998).

Fig. 3 further illustrates the importance of temperature variations by estimating the electric and magnetic field thresholds for the biophysical mechanism of voltage-gated membrane channels in a long cell (L_{cell} = 1 mm) (Weaver et al. 1998). The lowest curve (*dot-dashed line* independent of U_0) arises from thermal (Johnson-Nyquist) noise due to physical considerations only (Weaver and Astumian 1990), with a threshold E_{min} 2 × 10 ^{6} V/cm for a small bandwidth (f = 100 Hz) (Robertson and Astumian, 1991).

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FIGURE 3 Predicted threshold electric and magnetic fields for voltage-gated channels in a long cell for a prolonged exposure (t_{exp} = 10^4 s) relevant to weak 50-60-Hz fields (Weaver et al., 1998). This estimate is based on the predicted molecular (ionic) change due to a two-state channel model that competes with molecular changes (N and V); in real cells other processes (e.g., initiation of action potentials, electroporation) would occur at the larger electric field values and prevent the molecular change of a long exposure from occurring. The magnetic field case assumes an induced electric field using a current loop radius relevant to humans, r_{loop} = 0.3 m. The upper two bands indicate the temperature variation threshold, with the dark-shaded in vivo band assuming T_{dc} = 1-3°C and the light-shaded in vitro band having T_{dc} = 0.01-0.1°C. The "molecular shot noise" curve (*dashed line*) is based solely on fundamental molecular change fluctuations (Weaver et al., 1998). The lowest curve (*dot-dashed line*) does not involve molecular changes, but instead arises from Johnson-Nyquist noise, which involves purely physical quantities (Weaver and Astumian, 1990).

Equation 4 yields the next higher threshold curve (*dashed line*) (Astumian et al., 1995 ; Weaver et al., 1998), which for the long cell yields E_{min} as a function of U_0 (the smallest value is E_{min} 10 ^{4} V/cm; Weaver et al., 1998) and is about two orders of magnitude higher. The temperature variation threshold is represented by the top two bands, which are much higher than the fundamental molecular shot noise limit. These two bands are based on Eq. 7, including dc and ac temperature variations. The lower (*light-shaded*) band gives in vitro thresholds; the upper (*dark-shaded*) is the range for humans in vivo.

Table 1 shows a comparison between the predicted temperature variation threshold and measured electric field thresholds from in vitro experiments (Serpersu and Tsong, 1983 ; Graziana et al., 1990) on relatively simple biological systems, "voltage-gated" membrane-(Na,K)ATPase, where U_0 5kT. In both experiments, the observed thresholds are consistent with the predicted temperature variation threshold for T_{dc} in the range of 0.01-0.1°C.

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TABLE 1 Comparison of predicted and observed field magnitude temperature variation threshold for electric field alteration of membrane-associated (Na,K)ATPase activity in two relatively simple biological systems in vitro (Serpersu and Tsong, 1983 ; Graziana et al., 1990), for an exposure time of t_{exp} = 3600 s (erythrocytes) and 300 s (carrot protoplasts), using the range T_{dc} = 0.01-0.1°C

Biological sensory systems could escape the temperature variation threshold in two ways. First, evolutionary pressure may result in biochemical temperature compensation, for example, by utilizing two biochemical rates in series, each with nearly the same temperature coefficient. This is analogous to providing electrical circuit temperature compensation by using a voltage divider with matched elements. Second, neural processing may be involved to correct for sensed temperature variations.

For a nonsensory system (such as unorganized cells in vitro) to respond, however, field-induced changes must exceed the temperature variation threshold. Thus, if observed nonsensory effects are indeed due to weak 50-60-Hz field exposures in the presence of typical temperature sensitivities and typical temperature variations, then s nV, which implies involvement of an extraordinary biophysical mechanism, one that couples to the biochemical process orders of magnitude more strongly than voltage-gated membrane channels in a large cell (Fig. 3).

Moreover, in vitro conditions are artificially "quiet," in the sense that both temperature variations and other sources of molecular change competition are smaller than in in vivo conditions, or are absent altogether. This may allow in vitro observation of changes due to weak, extremely low frequency fields. For example, an impressive experiment using fibroblasts within a collagen matrix reported biochemical synthesis changes due to weak electric fields (McLeod et al., 1987). A strong coupling between the field and the cells may

be provided through electromechanical deformation of the charged extracellular matrix and cells, observable in vitro because normal tissue movement and associated molecular changes are absent (Vaughan and Weaver, 1998), as are normal physiological variations in regulatory biochemical levels. Not only are both N and V important, but also $V_{\text{in vivo}} > V_{\text{in vitro}}$, and other competing molecular changes further constrain the ability of weak 50-60-Hz fields to cause biological effects in vivo.

If an observed effect is to be convincingly interpreted as being due to an electric or magnetic field, then the apparently overwhelming molecular change due to realistic temperature variations must be understood. This requires controls that determine 1) the temperature sensitivity of the relevant biochemical process (e.g., biochemical synthesis, cell growth, enzyme activity, receptor binding) and 2) the order-of-magnitude temperature variations within the biological system. In most reports of weak 50-60-Hz effects, however, these controls are absent.

Reconciliation of reported in vitro observations is particularly challenging, as these experiments involve relatively unorganized cellular systems in comparison to the evolved multicellular systems that are believed to underlie electric and magnetic sensory systems. Sensory systems have a further advantage: they often respond in short times in which temperature variations are minimal and stochastic resonance can be of greatest benefit. However, for nonsensory systems and long exposures (both in vivo and in vitro), temperature variations are larger and stochastic resonance is ineffective. The suggestion (Bezrukov and Vodyanoy 1997b) that single cells might use stochastic resonance over very long times (up to 10^6 s) therefore appears unrealistic. Without an explicit analysis of competing molecular changes due to temperature variations, it is difficult to accept reports of effects associated with weak 50-60-Hz fields as being caused by these very small physical stimuli.

APPENDIX A

Heat transfer by conduction is diffusive, damping regulating temperature variations. We represent temperature regulation as a localized sinusoidal temperature source, with frequency f_T 10 ^{2} Hz. At a distance x away from the source, sinusoidal temperature variations of frequency f_T are reduced by a factor R_{damp} exp(x), where is the thermal diffusivity. Typically, 10 ^{3} cm^2/s.

Active cells in vivo are typically within x 20 μm from a blood vessel, yielding R_{damp} 0.99 (damping of 1%). Therefore, these cells experience essentially the full temperature variation of circulating blood, which in the core of humans varies by 1°C, and elsewhere is larger. This motivates our choice of the range 1-3°C for the in vivo temperature variation magnitude for exposure times greater than 100 s.

In contrast, in vitro values of x typically range from 0.1 to 1 cm, yielding 0.04-0.7 for R_{damp}. These values are consistent with microdegree ac variations in the bulk of a sample for short-duration (seconds) in vitro experiments. Long-duration (many minutes) experiments, however, will be subjected to significant dc temperature differences, but these are usually smaller than in in vivo systems; we use variations in the range 0.01 (T)$_{\text{dc}}$ 1°C to represent average, random dc temperature variations in vitro.

APPENDIX B

Competing changes arise from slight average (offset) random temperature differences T_{dc} that exist between the control and exposed systems (Fig. 1). Typically, T_{dc} 0.01°C in vitro, and T_{dc} 1°C in vivo. The associated average molecular change difference is

A sinusoidal (ac) temperature variation provides a simplified representation of temperature regulation. The sinusoidal contribution is obtained by using a second-order expansion of $J(T)$ around T_0 310 K:

For $U_0 = 8kT$ and $T = 310$ K, $_T$ 2.5×10^{-4} K $^{-2}$. The nonlinear temperature dependence of the Bolzmann factor in Eq. 1 results in rectification for an ac temperature variation at f_T 1 Hz. The cumulative average molecular change is

If regulation were perfect, then each system would experience this additional molecular change $_{V,ac}$, and the difference between the two systems would remain unchanged. However, if there are slight, random differences in temperature regulation, the resulting molecular change variability further obscures $_S$. For brevity, we consider only one source of ac temperature variability: slight differences in T_{ac}, the peak ac temperature, T_{ac} (Fig. 1). This yields fluctuations in molecular change,

For independent dc and ac temperature variations, the associated total molecular change is which is used in Eq. 7 to determine the temperature variation threshold (involving both shot noise and temperature variability, with the latter dominant; see Fig. 3).