Nonlinear response of the immune system to power-frequency magnetic fields

ANDREW A. MARINO,1 R. MICHAEL WOLCOTT,2 ROBERT CHERVENAK,2 FRANCES JOURD’HEUIL,2 ERIK NILSEN,1,3 AND CLIFTON FRILOT II4

Departments of 1Orthopaedic Surgery and 2Microbiology and Immunology, Louisiana State University Health Sciences Center, Shreveport 71130; and 3College of Engineering Sciences and 4Department of Bioengineering, Louisiana Tech University, Ruston, Louisiana 71272

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Marino, Andrew A., R. Michael Wolcott, Robert Chervenak, Frances Jourd’heuil, Erik Nilsen, and Clifton Frilot II. Nonlinear response of the immune system to power-frequency magnetic fields. Am J Physiol Regulatory Integrative Comp Physiol 279: R761–R768, 2000.—Studies of the effects of power-frequency electromagnetic fields (EMFs) on the immune and other body systems produced positive and negative results, and this pattern was usually interpreted to indicate the absence of real effects. However, if the biological effects of EMFs were governed by nonlinear laws, deterministic responses to fields could occur that were both real and inconsistent, thereby leading to both types of results. The hypothesis of real inconsistent effects due to EMFs was tested by exposing mice to 1 G, 60 Hz for 1–105 days and observing the effect on 20 immune parameters, using flow cytometry and functional assays. The data were evaluated by means of a novel statistical procedure that avoided averaging away oppositely directed changes in different animals, which we perceived to be the problem in some of the earlier EMF studies. The reliability of the procedure was shown using appropriate controls. In three independent experiments involving exposure for 21 or more days, the field altered lymphoid phenotype even though the changes in individual immune parameters were inconsistent. When the data were evaluated using traditional linear statistical methods, no significant difference in any immune parameter was found. We were able to mimic the results by sampling from known chaotic systems, suggesting that deterministic chaos could explain the effect of fields on the immune system. We conclude that exposure to power-frequency fields produced changes in the immune system that were both real and inconsistent.

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R761
and that interanimal measurement differences were due solely to stochastic processes. This assumption was sometimes made explicitly but more often was manifested by the choice of the statistical procedure employed to evaluate the data. In such a model, which we shall refer to as a linear stochastic model, a true response must be consistent from animal to animal. If it were the case, for example, that the field produced an increase in a parameter in one animal and a decrease or no change in a second animal, that result would violate either the assumption that the response was caused by the field or the assumption that the animals were identical.

In our concept of the link between EMF exposure and changes in the immune system, we assumed that if immune parameters were measured as a time series in each of a group of animals, the response of a given parameter could be an increase, decrease, or no change, depending on the animal. More particularly, the evolution of the time series could appear random but still contain a deterministic component due to the EMF. We chose parameters that could be measured in the same animal only once. Consequently, to test our hypothesis, we compared the average response of a group of exposed animals with that from a separate control group. We recognized that the mean of a large sample of exposed mice that each exhibited the putative inconsistent response would be similar to that of the controls even though, hypothetically, the field produced a deterministic response in some or even most of the exposed animals. In other words, in our conceptual model, an effect of the EMF would not be observed in a large sample because oppositely directed changes would be averaged away. A single small sample might reveal the putative effect (due to incomplete averaging), but tests on small samples lack statistical power. We therefore developed a statistical procedure that might permit us to infer the occurrence of irregular changes.

The likelihood approach allows differences in means from replicate series of exposed and control groups to be combined to test an overall hypothesis (5), in our case the hypothesis that changes occurred. The log-likelihood ratio of the $t$ statistic for a $t$-test between an exposed and control group is

$$L = 2n \ln \left( 1 + \frac{1}{2n - 2} t^2 \right)$$

where $n$ is the number of animals in each group. The distribution of $L$ is approximately $\chi^2$ with one degree of freedom. For $k$ pairs, the overall value of the test statistic, $L$, is $L = \sum_{i=1}^{k} L_i$, where $L_i$ is the ratio for $t$ for the $i^{th}$ parameter. $L$ also approximately follows the $\chi^2$ distribution, with $k$ degrees of freedom under the hypothesis of no treatment effect. Because $L$ is sensitive to the difference between the exposed and control groups but not to the direction of the difference, $L$ is suitable for testing a single overall hypothesis regarding occurrence of EMF-induced change in the $k$ replicates.

The occurrence of changes in the immune system due to exposure to 60-Hz magnetic fields was assessed on the basis of whether $L = \chi^2_{0.05}$, with $k = 3$ and $n = 5$. We chose a group size of five, because preliminary studies suggested it was large enough to characterize a population but small enough to permit the large number of planned measurements on each animal (see Immune Measurements). For small samples ($n = 5$), probabilities for $L$ determined from the $\chi^2$ distribution were slightly overestimated or underestimated, depending on $t$. These deviations from large-sample probabilities had no effect on our results (see DISCUSSION). Controls included sham-exposed mice paired with each exposed group and experiments in which both groups were sham exposed. Additionally, by sampling from two different mathematically defined chaotic systems, we studied whether the $L$ test was capable of recognizing that the systems actually differed. The occurrence of consistent change in the immune data (which would suggest the applicability of a linear model or a nonlinear nonchaotic model) was evaluated by combining the individual measurements in the three replicates before analysis ($L = \chi^2_{0.05}$, with $n = 15$). This procedure was equivalent to performing a $t$-test on the combined data.

More than 4,800 individual measurements of 20 different immune parameters were made. Of this total, ~15 measurements differed by more than five standard deviations from their respective means. The outliers were included in the analysis because we had no objective basis to exclude them.

**Exposure System**

Magnetic fields were produced using an arrangement of four square coils (21, 33). The coils were dipped in epoxy and wrapped with grounded, interrupted metal shielding. Four sets of four coils arranged in an octapole configuration (33) constituted a unit. One unit was used to produce magnetic fields. It was energized by a power supply consisting of an isolation transformer, autotransformer, and capacitors operated in series resonance at 60 Hz to eliminate powerline harmonics. The unit was designed using commercial software (MF3D, Electric Research and Management, Pittsburgh, PA) to produce magnetic fields that were homogeneous ($\pm 5\%$) throughout the region occupied by the mice with a negligible fringing field. The design specifications were verified by direct measurements (Bartington MAG-03, GMW, Redwood City, CA). Another unit was short circuited and used to house the control mice. The ambient 60-Hz field at that location averaged 0.4 mG and never exceeded 0.7 mG.

The exposure room was continuously maintained under temperature and humidity control with an unvarying 12:12-h light-dark cycle. Room air was replaced 15 times per hour with fresh air. A virtual instrument (Labview, National Instruments, Austin, TX) was created to continuously monitor and record room temperature, coil current, magnetic field, and current harmonic content.

**Animals**

This work was part of a larger study that employed both males and females. The experiments described here were performed on male mice, except where noted. C57BL/6 mice (Jackson Laboratories, Bar Harbor, MN), 6 wk old at arrival, were rested a minimum of 2 wk before use. The mice were housed in a totally nonmetallic environment. The water bottle was placed inside the cage to minimize differences in electrical potential between the mice and the water. The animal cages sat on plastic shelves that were wall mounted to prevent vibrational coupling between the coils and the cages. The mice were exposed continuously, except for the time needed to service the cages (~1 h/wk).

In separate experiments, mice were exposed to 1 G, 60 Hz for 1, 5, 10, 21, 49, and 105 days. In addition, to evaluate the reliability of the $L$ procedure, two sham experiments were performed in which all mice received the control treatment; these experiments used female mice. For each exposure period, three replicates were evaluated, each consisting of five exposed and five control mice. The 10 mice in a particular pair were killed (cervical dislocation) on the same morning, and the minimum time between deaths of any two pairs was 1 wk.
Flow cytometry. Spleen and thymus cells were obtained by gently dispersing the organs between glass slides, and bone marrow cells were obtained by removing and flushing both femurs with PBS. The cells were counted (Z1, Coulter, Hialeah, FL) and then resuspended at 10^7 cells per milliliter in PBS. The cells were counted (Z1, Coulter, Hialeah, FL). Cell populations were enumerated by staining with PK 136, for the NK1.1 antigen on NK cells, GK1.5, 2.43, 2C11, and anti-Thy1.2 for the respective CD4, CD8, CD3, and CD80 antigens on T cells, and anti-IgM, anti-IgD, and anti-CD45R for antigens on B cells. Antibodies were purified from hybridomas (American Type Culture Collection, Rockville, MD) or purchased (Pharmingen, San Diego, CA; Southern, Birmingham, AL). To prevent nonspecific binding, the cells were incubated with 50 μl of the appropriately diluted anti-Fc receptor antibody.

**Results.**

Immune Measurements

**Flow cytometry.** Spleen and thymus cells were obtained by gently dispersing the organs between glass slides, and bone marrow cells were obtained by removing and flushing both femurs with PBS. The cells were counted (Z1, Coulter, Hialeah, FL) and then resuspended at 10^7 cells per milliliter in PBS. The cells were counted (Z1, Coulter, Hialeah, FL). Cell populations were enumerated by staining with PK 136, for the NK1.1 antigen on NK cells, GK1.5, 2.43, 2C11, and anti-Thy1.2 for the respective CD4, CD8, CD3, and CD80 antigens on T cells, and anti-IgM, anti-IgD, and anti-CD45R for antigens on B cells. Antibodies were purified from hybridomas (American Type Culture Collection, Rockville, MD) or purchased (Pharmingen, San Diego, CA; Southern, Birmingham, AL). To prevent nonspecific binding, the cells were incubated with 50 μl of the appropriately diluted anti-Fc receptor antibody.

**Assays.** Cytotoxic T lymphocytes (CTL) were generated in a one-way mixed lymphocyte culture (MLC) by coculturing B6 spleen cells and gamma-irradiated AJJ spleen stimulator for 5 days. Proliferation was quantified (after 3 days’ incubation) using a commercial proliferation assay (CellTiter96 AQ, Promega, Madison, WI). The result was expressed as the ratio of absorption units obtained from stimulated and unstimulated culture (stimulation index). The lytic activity of CTL was measured by combining 50 μl of target cells with various numbers of effector cells to give a range of effector-to-target (E/T) ratios (2:1, 3:1, 6:1, 13:1, 25:1, 50:1, and 100:1) that each were assayed in triplicate. The specific lysis (SL) was calculated as SL = (E − S)/(M − S), where E and S were, respectively, the counts per minute released in the presence of the effector cells and the presence of medium alone, and M was the maximum value (determined by lysing the target cells with acetic acid). For determination of NK cell cytotoxicity, YAC-1 and P815 cells were used as positive and negative targets, respectively. YAC-1 and IL-2 cells were used as the respective positive and negative targets for the CTL generated in the MLC. For simplicity, the results are expressed in terms of a single predetermined E/T ratio for each assay.

The absence of numeric or functional data due to technical errors is shown in the tables.

**RESULTS**

Twenty immune parameters were measured in each of five mice exposed to 1 G for 105 days and in each of five sham-exposed control mice, and the mean ± SD for each parameter was determined. With the use of Equation I, I was calculated for each of the 20 comparisons between the two groups. The entire procedure was repeated twice, totaling three replicates, and 20 L values were computed by summing the corresponding constituent Ij (Table 1). We tested hypotheses concerning the occurrence of field-induced change by using the replicate means to assess whether L exceeded the critical value (P < 0.05) of the χ² distribution with three degrees of freedom and found six significant differences (Table 1).

The experiment was repeated five additional times, corresponding to exposures of 1, 5, 10, 21, and 49 days.

**Table 1. Immune parameters in mice exposed to 3 replicates to 1 G, 60 Hz, for 105 days.**

<table>
<thead>
<tr>
<th></th>
<th>Cellularity, no. of cells × 10⁷</th>
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<tbody>
<tr>
<td><strong>Spleen</strong></td>
<td></td>
</tr>
<tr>
<td>CD45</td>
<td>3</td>
</tr>
<tr>
<td>CD8</td>
<td>3</td>
</tr>
<tr>
<td>CD3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Thymus</strong></td>
<td></td>
</tr>
<tr>
<td>CD4</td>
<td>3</td>
</tr>
<tr>
<td>CD8</td>
<td>3</td>
</tr>
<tr>
<td>CD3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Bone marrow</strong></td>
<td></td>
</tr>
<tr>
<td>CD4</td>
<td>3</td>
</tr>
<tr>
<td>CD8</td>
<td>3</td>
</tr>
<tr>
<td>CD3</td>
<td>3</td>
</tr>
</tbody>
</table>

**Distribution,**

<p>| | |</p>
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<tr>
<th></th>
<th></th>
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<tbody>
<tr>
<td><strong>Spleen</strong></td>
<td></td>
</tr>
<tr>
<td>CD45</td>
<td>3</td>
</tr>
<tr>
<td>CD8</td>
<td>3</td>
</tr>
<tr>
<td>CD3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Thymus</strong></td>
<td></td>
</tr>
<tr>
<td>CD4</td>
<td>3</td>
</tr>
<tr>
<td>CD8</td>
<td>3</td>
</tr>
<tr>
<td>CD3</td>
<td>3</td>
</tr>
</tbody>
</table>

**Values are means ± SD. SI, stimulation index; CTLA, cytotoxic T lymphocyte assay [effector-to-target ratio (E/T) = 13:1]; NKA, natural killer cell cytotoxic assay (E/T = 25:1). E, exposed; C, control; l, log-likelihood ratio of statistic; L, sum of the ratios. Absence of data indicated by blank spaces. *P < 0.05.**
After exposure for 49 days, statistically significant differences in five immune parameters were found (Table 2), and seven such differences were found after exposure for 21 days (Table 3). Two significant changes were found after exposure for 1 or 10 days, and 3 significant changes were found after exposure for 5 days (data not shown).

To explore the possibility that the relatively large numbers of significant differences were somehow a by-product of the novel procedure adopted for assessing occurrence of EMF-induced change, the experiment was performed two additional times under conditions employed in the experiments described above, except that the field was not applied to the putatively exposed group in either sham study. The sham exposure lasted 21 days in 1 case and 75 days in the other. Each sham-exposed group and its corresponding control were identical in every respect that was known or suspected as potentially capable of affecting the immune system. We performed the 20 L tests in each of the two experiments and found one significant difference in each experiment. Table 4 lists the data for the 21-day sham; the data for the 75-day sham are not shown.

The statistically significant differences observed in all field and sham experiments are summarized in Table 5, and the cumulative frequency of the significant results as a function of L is shown in Fig. 1.

An additional check on the reliability of the L test was performed. We reasoned that if sets of samples were drawn from two different known chaotic systems, the analytic procedure ought to be capable of recognizing that the systems differed in at least some cases where conventional linear analysis failed to do so. We therefore formed three replicates (n = 5) from the logistic equations \(x_k = 3.89x_{k-1}(1-x_{k-1})\) and \(y_k = 3.99y_{k-1}(1-y_{k-1})\), with \(x_0 = 0.4, k = 1, 2, \ldots, 100\), both of which are chaotic but fully deterministic, and analyzed the results using L. A typical result is shown in Table 6. The procedure resulted in recognition that the two populations actually differed (L = 7.95, P < 0.05), even though no difference was found when the 15 individual samples were combined before computing L (L = 2.49, P > 0.05).

### DISCUSSION

#### Observation of Real Change

The small-sample replicate-structure procedure based on the log-likelihood ratio statistic for the t-test was capable of detecting any form of change induced by the field, whether consistent or inconsistent. In this regard, our study differed from previous EMF-bioeffects studies, most of which employed statistical designs that were insensitive to inconsistent change. We found that in each of two sham experiments, chance alone produced only one significant comparison in 20 tests, which was the expected result. The increased frequency of significant differences observed in the groups exposed for 1, 5, and 10 days could also, argue,
Table 3. Immune parameters in mice exposed in 3 replicates to 1 G, 60 Hz, for 21 days

<table>
<thead>
<tr>
<th>Replicate</th>
<th>E</th>
<th>C</th>
<th>I</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cellularity, no. of cells x10^7</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>1</td>
<td>12.4 ± 0.9</td>
<td>10.2 ± 0.5</td>
<td>12.76</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.9 ± 1.1</td>
<td>12.1 ± 1.9</td>
<td>4.93</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>11.9 ± 1.0</td>
<td>12.2 ± 1.5</td>
<td>0.16</td>
</tr>
<tr>
<td>Thymus</td>
<td>1</td>
<td>4.9 ± 1.1</td>
<td>5.0 ± 1.1</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.8 ± 2.2</td>
<td>4.2 ± 2.4</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.4 ± 1.8</td>
<td>4.2 ± 1.2</td>
<td>0.09</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>1</td>
<td>3.0 ± 0.4</td>
<td>3.0 ± 0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.8 ± 0.2</td>
<td>2.9 ± 0.6</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.2 ± 0.2</td>
<td>4.4 ± 0.3</td>
<td>1.55</td>
</tr>
</tbody>
</table>

**Distribution, %**

| Spleen | | | | |
| CD45 | 1 | 2.6 ± 0.9 | 2.4 ± 0.6 | 0.22 |
| | 2 | 2.6 ± 0.6 | 2.8 ± 0.4 | 0.49 |
| | 3 | 3.2 ± 1.0 | 2.8 ± 1.0 | 0.70 |
| IgM+ | 1 | 59.0 ± 2.8 | 62.2 ± 1.3 | 5.06 |
| | 2 | 69.8 ± 3.8 | 59.2 ± 7.0 | 3.74 |
| | 3 | 55.2 ± 11.1 | 61.2 ± 1.3 | 1.42 |
| IgM+IgD- | 1 | 10.2 ± 1.3 | 9.4 ± 0.6 | 1.82 |
| | 2 | 10.4 ± 2.2 | 10.4 ± 1.5 | 0 |
| | 3 | 10.8 ± 3.8 | 12.8 ± 1.5 | 1.20 |
| IgM+IgD+ | 1 | 48.4 ± 4.9 | 54.2 ± 2.3 | 5.45 |
| | 2 | 52.8 ± 2.7 | 49.2 ± 5.4 | 2.01 |
| | 3 | 46.0 ± 7.8 | 50.0 ± 2.0 | 1.22 |
| CD90+CD3+ | 1 | 19.8 ± 0.7 | 19.0 ± 2.2 | 0.84 |
| | 2 | 16.4 ± 0.8 | 17.0 ± 2.6 | 0.28 |
| | 3 | 13.0 ± 3.0 | 14.2 ± 1.5 | 0.52 |
| NK1.1 | 1 | 3.4 ± 1.0 | 3.1 ± 0.6 | 0.54 |
| | 2 | 3.3 ± 0.5 | 3.7 ± 0.4 | 3.10 |
| | 3 | 3.7 ± 1.3 | 4.6 ± 0.5 | 1.97 |
| Marrow | | | | |
| CD45 | 1 | 5.4 ± 1.7 | 7.8 ± 1.3 | 5.88 |
| | 2 | 11.6 ± 4.2 | 11.4 ± 3.1 | 0.01 |
| | 3 | 8.8 ± 3.8 | 9.8 ± 2.0 | 0.34 |
| IgM+ | 1 | 11.2 ± 2.2 | 13.0 ± 0.7 | 3.29 |
| | 2 | 12.6 ± 1.3 | 9.8 ± 2.8 | 4.16 |
| | 3 | 7.2 ± 3.4 | 11.8 ± 1.7 | 5.27 |
| IgM+IgD- | 1 | 8.4 ± 2.3 | 12.2 ± 2.3 | 6.20 |
| | 2 | 6.6 ± 3.0 | 8.2 ± 2.8 | 0.93 |
| | 3 | 4.2 ± 2.6 | 4.8 ± 0.5 | 0.18 |
| IgM+IgD+ | 1 | 6.0 ± 1.9 | 6.2 ± 0.4 | 0.07 |
| | 2 | 8.0 ± 1.0 | 5.6 ± 1.1 | 9.42 |
| | 3 | 4.0 ± 1.7 | 7.0 ± 1.4 | 7.33 |
| Thymus | | | | |
| CD90+CD3+ | 1 | 9.4 ± 1.3 | 9.2 ± 1.3 | 0.07 |
| | 2 | 22.0 ± 11.9 | 14.0 ± 4.5 | 2.19 |
| | 3 | 15.8 ± 2.9 | 12.8 ± 1.0 | 4.03 |
| CD4+CD8- | 1 | 10.8 ± 0.8 | 10.4 ± 1.7 | 0.28 |
| | 2 | 23.4 ± 6.8 | 18.8 ± 4.6 | 1.80 |
| | 3 | 11.0 ± 1.8 | 10.5 ± 0.6 | 0.36 |
| CD4+CD8+ | 1 | 9.4 ± 2.2 | 7.3 ± 1.1 | 3.54 |
| | 2 | 3.5 ± 2.8 | 2.1 ± 1.2 | 1.17 |
| | 3 | 1.9 ± 0.5 | 1.0 ± 0.3 | 8.26 |
| CD4+CD8- | 1 | 69.0 ± 3.6 | 74.6 ± 3.2 | 6.10 |
| | 2 | 67.0 ± 12.2 | 74.2 ± 5.4 | 1.67 |
| | 3 | 82.0 ± 2.0 | 84.8 ± 1.0 | 5.64 |

**Function**

| SI (ratio of cell numbers) | | | | |
| 1 | 2.4 ± 0.5 | 2.8 ± 0.2 | 2.86 |
| 2 | 2.3 ± 0.4 | 2.0 ± 0.6 | 1.16 |
| 3 | | | |
| CTLa, % | 1 | 43.6 ± 12.3 | 53.6 ± 17.0 | 1.32 |
| | 2 | 61.8 ± 2.5 | 59.8 ± 9.4 | 0.29 |
| | 3 | | | |
| NKA, % | 1 | 20.6 ± 5.8 | 18.6 ± 4.3 | 0.47 |
| | 2 | 28.8 ± 4.1 | 34.5 ± 6.1 | 3.04 |
| | 3 | 22.8 ± 7.6 | 23.3 ± 1.5 | 0.02 |

Values are means ± SD. *P < 0.05.

NONLINEAR FIELD EFFECTS
Table 4. Immune parameters in 3 replicates of sham-exposed mice

<table>
<thead>
<tr>
<th>Replicate</th>
<th>E</th>
<th>C</th>
<th>L</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen 1</td>
<td>7.46 ± 1.78</td>
<td>8.44 ± 1.09</td>
<td>1.291</td>
<td>4.649</td>
</tr>
<tr>
<td>2</td>
<td>10.34 ± 2.28</td>
<td>8.20 ± 2.60</td>
<td>2.149</td>
<td>7.932</td>
</tr>
<tr>
<td>3</td>
<td>10.14 ± 2.32</td>
<td>8.84 ± 1.69</td>
<td>1.299</td>
<td>7.572</td>
</tr>
<tr>
<td>Thymus 1</td>
<td>2.96 ± 1.63</td>
<td>5.38 ± 1.29</td>
<td>6.160</td>
<td>5.578</td>
</tr>
<tr>
<td>2</td>
<td>6.20 ± 2.62</td>
<td>4.84 ± 2.04</td>
<td>1.299</td>
<td>4.649</td>
</tr>
<tr>
<td>3</td>
<td>5.74 ± 1.48</td>
<td>5.36 ± 2.26</td>
<td>0.123</td>
<td>0.986</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>1</td>
<td>4.12 ± 0.70</td>
<td>4.26 ± 1.22</td>
<td>0.061</td>
</tr>
<tr>
<td>2</td>
<td>3.26 ± 0.44</td>
<td>3.65 ± 0.08</td>
<td>4.391</td>
<td>4.907</td>
</tr>
<tr>
<td>3</td>
<td>3.94 ± 0.58</td>
<td>3.72 ± 0.56</td>
<td>0.455</td>
<td>0.455</td>
</tr>
</tbody>
</table>

Table 5. The L statistic for comparisons involving the effect of exposure to 1 G, 60 Hz on immune parameters in mice

<table>
<thead>
<tr>
<th>Function</th>
<th>Sham</th>
<th>Electromagnetic Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity, no. of cells × 10^7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>17.85</td>
<td>8.34 10.36</td>
</tr>
<tr>
<td>Thymus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone Marrow</td>
<td></td>
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</tr>
</tbody>
</table>

Value of L for all pairwise comparisons for which \( P < 0.05 (L > 7.83) \) is listed.

The set of parameters was affected by EMF exposure for 105, 49, and 21 days (\( P < 0.05 \)) in independent experiments and was probably affected by exposure for 5 days \( (P=0.08) \) and for 1 and 10 days \( (P=0.26) \). The data were fundamentally consistent because, in each case, they rationalized the inference of a cause-and-effect relationship between fields and change in the immune system and, more particularly, the inference that biological transduction of the field must have occurred.

Explanation of Change

The determinism reflected in the results (Tables 1–3) cannot be explained on the basis of a linear stochastic model, thereby indicating the need for some kind of nonlinear model. The body contains numerous nonlinear sensory and effector systems that function more or less predictably. The data presented here suggest that, at least in connection with the link between power-frequency EMFs and change in lymphoid phenotype, there also exist nonlinear systems that do not function with the same kind of predictability manifested by known nonlinear systems. Some nonlinear
mathematical and physical systems exhibit a form of behavior in which small differences in initial conditions can dramatically affect the evolution of the system with the result that, after an initial period, the system's behavior cannot be predicted with any more reliability than that of a guess. Such sensitivity to initial conditions has been termed deterministic chaos (28). It is possible that a model based on chaotic nonlinearity could explain our results (Table 6). The nature of the nonlinearity, however, cannot be established unequivocally, because time series data from individual animals were not obtained.

It is worthwhile to speculate about why the effects of 60-Hz magnetic fields on the immune system happened to be inconsistent. Predictability of input-output relationships in the body's sensory and effector systems is mediated by mechanisms that were shaped by natural selection. From an evolutionary viewpoint, however, power-frequency magnetic fields were a negligible factor. A consistent response would serve no evolutionary purpose, and, consequently, a mechanism capable of producing a consistent response probably did not evolve. From this perspective, the cellular basis of EMF-induced effects, such as those involving the immune system, seem better understood as a vulnerability in the body's sensory or regulatory systems inherent in the specific designs of those systems that were selected by nature.

Finally, this study was designed to show that interaction of power-frequency EMFs with biological receptors could cause changes that were both real and inconsistent, thereby showing that unreality could not validly be inferred from inconsistency. The question of the possible biophysical mechanism responsible for the interaction has been considered by others (7, 8, 12) and was not addressed here. The further question of the biological significance of inconsistent change in the immune system was similarly not addressed here.

Perspectives

An important goal of modern science is to understand the mechanisms that mediate particular observations. But observations are determined not simply by mechanisms, but also by the dynamic law that governs them. Assumption of a linear theory is often sufficient to explain physiological data. However, many previous studies suggested that this is not the case for changes caused by EMFs. We showed directly that only a nonlinear approach could explain our data. Thus, by generalizing the model to allow for nonlinear dynamic laws, it was possible to understand how EMFs could cause real physiological effects. Nonlinear effects could be as important as linear effects in predisposing an organism toward disease. Consequently, the existence of nonlinear physiological changes due to EMFs may necessitate reevaluation of assessments of potential public-health risks that were based on linear effects (2, 4, 30).

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Table 6. Data obtained from random sampling from chaotic systems defined by logistic equations

<table>
<thead>
<tr>
<th>Replicate No.</th>
<th>x_k</th>
<th>y_k</th>
<th>l</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.698 ± 0.350</td>
<td>0.524 ± 0.251</td>
<td>2.548</td>
<td>7.945*</td>
</tr>
<tr>
<td>2</td>
<td>0.438 ± 0.339</td>
<td>0.685 ± 0.217</td>
<td>5.391</td>
<td>0.006</td>
</tr>
<tr>
<td>3</td>
<td>0.569 ± 0.285</td>
<td>0.577 ± 0.214</td>
<td>0.006</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Values are means ± SD. Data were normalized so that the mean of 1 group in each replicate (the normal control group) was 1.0. No difference was found when L was computed directly from the 15 individual values without regard to replicate structure (L = 2.49, P > 0.05). *P < 0.05, x_k, y_k. Each represents the state of a dynamic system defined by a logistic equation at a particular discrete time, k.
REFERENCES


