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

**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 inter-
preted 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 re-
sults. 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 in-
volving exposure for 21 or more days, the field altered lym-
phoid phenotype even though the changes in individual im-
mune 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 con-
clude that exposure to power-frequency fields produced
changes in the immune system that were both real and
inconsistent.

chaos; electromagnetic fields; powerlines

WHETHER ELECTROMAGNETIC FIELDS (EMFs) from electrical
transmission and distribution systems constitute a
health hazard has been disputed since the issue first
became prominent (18). Epidemiological studies impli-
cated EMFs in many different diseases (23, 27, 29, 32),
but theoretical approaches failed to unambiguously
rationalize any correlations (1, 11, 31), and laboratory
bioassay studies aimed at understanding the range
and potential seriousness of EMF-induced bioeffects
seemed always to result in inconsistency. EMFs al-
tered the immune system in some studies (22), but not
in others (15). EMFs affected the rate of release of Ca^{2+}
from brain tissue (6), but others were unable to repro-
duce the effect (3). EMFs caused skeletal growth ab-
normalities in chicks (9), but the same model system in
the hands of other investigators yielded negative re-
sults (14). Sometimes EMFs affected growth rate of
animals (19), but not in other cases (24). EMFs affected
transcription (10) or not (26) in seemingly identical
experiments performed by different investigators.
EMF studies were similarly inconsistent in other ar-
eas, including effects on melatonin levels, stress reac-
tions, behavior, and cell growth. Whether the result of
any particular EMF experiment would be positive or
negative was, and continues to be, unpredictable.

Committees of experts assumed that only consistent
data could evidence an interaction between fields and
tissue and, finding no such data, concluded there were
no real EMF bioeffects and therefore no reasonable
possibility of health risks due to EMF exposure (2, 4,
30). It occurred to us, however, that the assumption
was dubious, because nonlinear chaotic systems can be
both deterministic and unpredictable (13, 25, 28). We
previously presented indirect experimental evidence
suggesting that the effects of long-term exposure to
60-Hz EMFs on the growth rate of mice were consistent
with the theory of deterministic chaos (16, 19, 20).

Our goal here was to show directly that exposure to
power-frequency EMFs could cause biological effects
that were both real and inconsistent. We chose lym-
phoid phenotype as the endpoint because earlier work
suggested that the immune system was a key mecha-
nism linking field exposure and disease (17).

METHODS

Experimental Design

In most previous EMF bioeffects studies, it was assumed
that any response of an animal to the field would be linear

---

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

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

where n is the number of animals in each group. The distribution of l is approximately χ^2 with one degree of freedom. For k pairs, the overall value of the test statistic, L, is $L = \sum_1^k l_i$, where l_i is the ratio for t for the ith parameter. L also approximately follows the χ^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 $L = \chi^2_{k,0.005}$, 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 χ^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 ($L = \chi^2_{1,0.05}$, 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 ($\sim$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.

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 staining buffer (PBS, 2% fetal bovine serum, 1 g/l sodium azide), and populations of interest were identified by two-color flow cytometric analysis using fluorescein isothyocyanate and phycoerythrin (Epics Profile II, 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 CD90 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 A/J 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 spleen NK cells was enhanced by culturing spleen cells for 18 h in medium containing interleukin-2 (800 units/ml) (34).

^{51}Cr-labeled target cells were combined 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 were each 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-4 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 1*, l 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 l_i (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 χ^2 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 in 3 replicates to 1 G, 60 Hz, for 105 days*

	Replicate	E	C	l	L
Cellularity, no. of cells $\times 10^7$					
Spleen	1	11.1 ± 1.3	10.6 ± 1.6	0.36	
	2	9.3 ± 0.9	10.7 ± 2.4	1.57	5.75
	3	13.7 ± 3.0	11.0 ± 1.1	3.82	
Thymus	1	3.2 ± 0.9	3.6 ± 0.9	0.53	
	2	2.0 ± 0.5	3.2 ± 0.6	9.12	10.36*
	3	3.4 ± 1.0	3.0 ± 0.7	0.71	
Bone marrow	1	2.3 ± 0.9	1.0 ± 0.6	2.03	
	2	2.7 ± 0.4	2.7 ± 1.1	0.03	2.87
	3	3.7 ± 1.2	4.4 ± 1.6	0.81	
Distribution, %					
Spleen					
CD45	1	3.7 ± 1.4	3.9 ± 0.4	0.12	
	2	3.9 ± 0.5	3.0 ± 0.6	5.53	11.34*
	3	8.0 ± 0.8	7.30 ± 0.7	5.68	
IgM+	1	62.2 ± 3.0	64.8 ± 2.2	1.24	
	2	64.0 ± 0.7	65.4 ± 3.0	1.27	3.06
	3	59.2 ± 4.0	57.6 ± 3.6	0.55	
IgM+IgD−	1	20.6 ± 1.5	20.75 ± 1.26	0.032	
	2	14.6 ± 1.1	14.80 ± 1.10	0.100	1.664
	3	21.8 ± 1.9	23.20 ± 1.92	1.532	
IgM+IgD+	1	41.0 ± 3.7	43.0 ± 2.2	1.08	
	2	48.6 ± 0.9	51.6 ± 2.0	7.99	9.86*
	3	43.6 ± 2.5	41.8 ± 4.3	0.79	
CD90+CD3+	1	3.5 ± 0.5	3.1 ± 0.5	1.84	
	2	12.6 ± 1.8	11.3 ± 1.3	1.86	5.02
	3	3.3 ± 2.2	2.3 ± 0.4	1.31	
NK1.1	1				
	2	0.9 ± 0.3	0.7 ± 0.2	0.99	5.17
	3	0.9 ± 0.4	1.4 ± 0.4	4.18	
Marrow					
CD45	1	7.4 ± 2.3	7.2 ± 1.5	0.02	
	2	7.8 ± 1.3	8.4 ± 2.1	0.37	1.66
	3	8.2 ± 1.7	7.0 ± 1.9	1.28	
IgM+	1	13.6 ± 1.7	15.2 ± 1.3	2.89	
	2	11.8 ± 1.8	11.0 ± 1.0	0.91	4.43
	3	17.2 ± 2.6	15.2 ± 5.2	0.63	
IgM+IgD−	1	9.0 ± 1.2	9.50 ± 0.58	0.69	
	2	5.8 ± 2.0	5.60 ± 1.14	0.04	5.76
	3	15.0 ± 5.0	9.40 ± 2.07	5.03	
IgM+IgD+	1	8.4 ± 1.1	9.5 ± 1.3	2.10	
	2	7.6 ± 0.6	6.4 ± 0.6	9.16	11.46*
	3	9.7 ± 0.6	9.0 ± 2.9	0.19	
Thymus					
CD90+CD3+	1	6.8 ± 1.9	7.2 ± 0.5	0.26	
	2	12.6 ± 2.9	9.8 ± 5.3	1.28	1.84
	3	9.0 ± 2.1	9.6 ± 1.7	0.30	
CD4+CD8−	1	7.3 ± 1.9	7.9 ± 0.4	0.45	
	2	9.4 ± 1.8	10.5 ± 0.9	1.71	2.38
	3	10.8 ± 2.3	11.9 ± 5.4	0.22	
CD4−CD8+	1	1.6 ± 0.6	1.5 ± 0.6	0.09	
	2	1.8 ± 0.4	2.2 ± 0.4	2.23	3.60
	3	8.6 ± 1.5	7.2 ± 2.6	1.28	
CD4+CD8+	1	86.6 ± 0.9	85.8 ± 0.5	3.07	
	2	84.0 ± 0.7	83.2 ± 1.1	2.11	5.23
	3	74.8 ± 2.8	75.4 ± 6.4	0.05	
Function					
SI (ratio of cell numbers)	1	2.0 ± 0.6	1.4 ± 0.2	4.48	
	2	2.4 ± 0.5	2.1 ± 0.2	1.44	7.83*
	3	1.4 ± 0.2	1.6 ± 0.3	1.90	
CTLa, %	1	38.8 ± 6.1	40.0 ± 4.9	0.13	
	2	43.8 ± 7.1	51.2 ± 6.3	3.21	3.34
	3				
NKa, %	1	35.4 ± 5.0	29.5 ± 8.5	1.96	
	2	26.8 ± 2.6	22.6 ± 1.5	8.00	13.97*
	3	21.8 ± 3.8	14.6 ± 7.2	4.02	

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 t 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 $x_0 = y_0 = 0.4$, $k = 1, 2, \ldots, 100$, 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 inconsistent or consistent. 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, argu-

Table 2. *Immune parameters in mice exposed in 3 replicates to 1 G, 60 Hz, for 49 days*

	Replicate	E	C	l	L
		Cellularity, no. of cells $\times 10^7$			
Spleen	1	12.0 ± 3.2	11.1 ± 1.2	0.45	
	2	11.0 ± 0.9	11.7 ± 0.8	1.28	1.96
	3	10.5 ± 1.2	10.2 ± 0.9	0.24	
Thymus	1	4.0 ± 1.7	5.9 ± 0.6	5.31	
	2	5.5 ± 0.7	6.6 ± 1.3	2.94	8.34*
	3	5.3 ± 0.6	5.2 ± 0.6	0.09	
Bone marrow	1	2.9 ± 0.3	3.2 ± 0.6	0.93	
	2	3.7 ± 0.6	4.0 ± 0.7	0.34	1.47
	3	4.5 ± 0.4	4.5 ± 0.3	0.19	
		Distribution, %			
Spleen					
CD45	1	2.8 ± 0.4	3.0 ± 0.7	0.35	
	2	3.2 ± 0.8	3.3 ± 0.6	0.08	0.43
	3	2.6 ± 0.6	2.6 ± 0.6	0	
IgM+	1	56.0 ± 10.8	65.0 ± 2.1	3.50	
	2	64.8 ± 3.0	63.3 ± 0.6	0.86	4.36
	3	65.6 ± 2.0	65.6 ± 0.6	0	
IgM+IgD−	1	7.9 ± 1.4	10.5 ± 0.9	9.35	
	2	10.1 ± 1.4	9.2 ± 0.1	1.34	12.67*
	3	9.8 ± 0.6	10.4 ± 0.8	1.98	
IgM+IgD+	1	45.8 ± 11.5	51.8 ± 2.8	1.49	
	2	53.8 ± 3.6	53.7 ± 2.1	0.00	1.51
	3	52.8 ± 2.6	52.6 ± 2.1	0.02	
CD90+CD3+	1	13.4 ± 3.8	13.6 ± 2.2	0.01	
	2	15.4 ± 3.2	14.7 ± 0.6	0.19	0.56
	3	17.6 ± 1.7	18.4 ± 2.9	0.35	
NK1.1	1	2.7 ± 0.4	3.5 ± 0.4	8.42	
	2	3.4 ± 0.2	3.4 ± 0.3	0.01	9.28*
	3	3.3 ± 0.2	3.1 ± 0.5	0.84	
Marrow					
CD45	1	10.8 ± 4.9	12.6 ± 3.1	0.59	
	2	13.1 ± 2.1	13.7 ± 0.3	0.27	0.86
	3	13.5 ± 1.5	13.4 ± 1.2	0.00	
IgM+	1	9.2 ± 5.1	10.6 ± 2.4	0.38	
	2	12.2 ± 0.8	12.0 ± 1.0	0.12	1.72
	3	12.4 ± 0.9	11.6 ± 1.5	1.21	
IgM+IgD−	1	5.0 ± 2.6	5.2 ± 1.6	0.03	
	2	6.8 ± 1.9	7.7 ± 2.9	0.35	1.05
	3	8.4 ± 3.8	6.8 ± 3.0	0.67	
IgM+IgD+	1	5.0 ± 2.9	5.6 ± 1.1	0.23	
	2	6.8 ± 0.8	6.3 ± 0.6	0.89	2.79
	3	6.8 ± 1.1	6.0 ± 1.0	1.67	
Thymus					
CD90+CD3+	1	12.6 ± 3.3	13.8 ± 4.6	0.28	
	2	16.4 ± 2.5	14.3 ± 3.5	1.19	7.86*
	3	17.4 ± 2.2	13.6 ± 2.3	6.39	
CD4+CD8−	1	12.8 ± 1.8	14.4 ± 2.8	1.36	
	2	19.4 ± 7.9	24.0 ± 4.4	1.04	5.28
	3	11.0 ± 1.0	12.2 ± 1.3	2.88	
CD4−CD8+	1	1.9 ± 0.9	1.7 ± 0.6	0.36	
	2	1.4 ± 0.2	1.6 ± 0.1	2.88	5.21
	3	1.7 ± 0.2	1.6 ± 0.2	1.13	
CD4+CD8+	1	81.2 ± 3.4	80.8 ± 2.6	0.05	
	2	76.2 ± 7.7	70.7 ± 4.0	1.55	2.85
	3	83.6 ± 1.1	82.8 ± 1.3	1.25	
		Function			
SI (ratio of cell numbers)	1	2.6 ± 0.4	2.6 ± 0.2	0.04	
	2	2.9 ± 0.3	3.0 ± 0.1	0.26	1.68
	3	1.4 ± 0.2	1.5 ± 0.2	1.38	
CTLa, %	1	39.4 ± 8.3	30.0 ± 10.3	2.73	
	2	67.2 ± 6.9	60.7 ± 5.0	2.31	5.20
	3	77.0 ± 2.8	78.2 ± 4.6	0.16	
NKa, %	1	18.6 ± 4.7	25.8 ± 6.0	4.44	
	2	19.0 ± 7.4	25.7 ± 0.6	2.60	8.14*
	3	31.6 ± 1.7	30.0 ± 3.3	1.1	

Values are means ± SD. *$P < 0.05$.

Table 3. *Immune parameters in mice exposed in 3 replicates to 1 G, 60 Hz, for 21 days*

	Replicate	E	C	l	L
Cellularity, no. of cells $\times 10^7$					
Spleen	1	12.4 ± 0.9	10.2 ± 0.5	12.76	
	2	9.9 ± 1.1	12.1 ± 1.9	4.93	17.85*
	3	11.9 ± 1.0	12.2 ± 1.5	0.16	
Thymus	1	4.9 ± 1.1	5.0 ± 1.1	0.02	
	2	3.8 ± 2.2	4.2 ± 2.4	0.11	0.23
	3	4.4 ± 1.8	4.2 ± 1.2	0.09	
Bone marrow	1	3.0 ± 0.4	3.0 ± 0.5	0	
	2	2.8 ± 0.2	2.9 ± 0.6	0.25	1.79
	3	4.2 ± 0.2	4.4 ± 0.3	1.55	
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	1.41
	3	3.2 ± 1.0	2.8 ± 1.0	0.70	
IgM+	1	59.0 ± 2.8	62.2 ± 1.3	5.06	
	2	63.6 ± 3.8	59.2 ± 7.0	1.74	8.23*
	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	3.02
	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	8.68*
	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	1.64
	3	13.0 ± 3.0	14.2 ± 1.8	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	5.60
	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	6.23
	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	12.71*
	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	7.31
	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	16.82*
	3	4.0 ± 1.4	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	6.29
	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	2.44
	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	12.97*
	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	13.42*
	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	4.01
	3				
CTLa, %	1	43.6 ± 12.3	53.6 ± 17.0	1.32	
	2	61.8 ± 2.5	59.8 ± 9.4	0.29	1.61
	3				
NKa, %	1	20.6 ± 5.8	18.6 ± 4.3	0.47	
	2	28.8 ± 4.1	34.5 ± 6.1	3.04	3.53
	3	22.8 ± 7.6	23.3 ± 1.5	0.02	

Values are means $\pm$ SD. *$P < 0.05$.

ably, be attributed to chance. For example, there was a small possibility ($P = 0.08$, binomial theorem) that all three significant comparisons at 5 days were due to stochastic processes unrelated to the presence of the field. For exposures ≥ 21 days, however, it is highly implausible that the observed frequencies of statistically significant differences (Fig. 1) occurred by chance.

The question arose whether the significant differences could somehow be due to the use of the χ^2 distribution, because L for small samples ($n = 5$) is not precisely χ^2. It can be shown that small-sample P is overestimated when $t < 2.05$ (ranging from 20% for $t = 0.1$ to 1% for $t = 2.0$); otherwise it is underestimated. Application of the appropriate correction factor to each l_i in the study did not alter the results. Furthermore, as mentioned, the overall reliability of the L procedure was directly verified in each of two sham studies. We conclude, therefore, that, at least in the experiments involving exposure for 21, 49, and 105 days, the increased rate of significant comparisons reliably indicated that the EMFs were transduced into biological signals that ultimately resulted in effects involving the immune system, notwithstanding that the data were, from a particular viewpoint, inconsistent (see *Nature of Change*).

Nature of Change

Our experimental design avoided testing hypotheses in individual replicates in favor of evaluating a single overall hypothesis for each parameter in a particular experiment. Nevertheless, it can be seen that the relative value of the means of corresponding exposed and control groups varied from replicate to replicate (Tables 1–4). To illustrate the fundamental role of this inconsistency in manifesting the determinism produced by the field, we reanalyzed the data without using the replicate structure design that was intended to obviate the problem that we perceived at the inception of this study (averaging away real effects). This was accomplished by computing L directly from the 15 exposed and 15 control mice and evaluating for statistical significance based on the χ^2 distribution with one degree of freedom. This procedure produced no significant effects in any of the instances in which effects were noted initially (Fig. 1).

Given the occurrence of an effect involving a particular immune parameter in a particular experiment, it would be reasonable to expect that the same parameter would be affected at other exposure times. Although reasonable, such expectations are not logical necessities and, in fact, often were not realized. In most cases, when a particular parameter was significantly affected after a specific exposure duration, the same parameter was not affected at either longer or shorter exposure times (Table 5). This phenomenon constitutes a second kind of inconsistency in the data.

Although the results reported here were inconsistent in the two senses just described, they were consistent in a more fundamental sense. The results showed that the immune system, as characterized by a particular

Table 4. *Immune parameters in 3 replicates of sham-exposed mice*

	Replicate	E	C	l	L
Cellularity, no. of cells $\times 10^7$					
Spleen	1	7.46 ± 1.78	8.44 ± 1.09	1.291	
	2	10.34 ± 2.28	8.20 ± 2.60	2.149	4.649
	3	10.14 ± 2.32	8.84 ± 1.69	1.209	
Thymus	1	2.96 ± 1.63	5.38 ± 1.29	6.160	
	2	6.20 ± 2.62	4.64 ± 2.04	1.289	7.572
	3	5.74 ± 1.48	5.36 ± 2.26	0.123	
Bone marrow	1	4.12 ± 0.70	4.26 ± 1.22	0.061	
	2	3.26 ± 0.44	3.68 ± 0.08	4.391	4.907
	3	3.94 ± 0.58	3.72 ± 0.56	0.455	
Distribution, %					
Spleen					
CD45	1	3.84 ± 0.85	4.02 ± 0.86	0.138	
	2	6.56 ± 0.86	6.96 ± 1.11	1.444	3.112
	3	6.62 ± 0.95	6.10 ± 0.35	1.530	
IgM+	1	51.74 ± 8.32	53.52 ± 2.76	0.254	
	2	52.40 ± 1.68	51.58 ± 1.75	0.686	1.038
	3	56.52 ± 6.59	55.58 ± 3.51	0.098	
IgM+IgD−	1	18.06 ± 2.56	17.18 ± 1.45	0.544	
	2				1.127
	3	16.30 ± 2.54	15.26 ± 2.19	0.583	
IgM+IgD+	1	37.36 ± 1.65	39.34 ± 1.76	3.506	
	2				3.518
	3	41.70 ± 3.77	41.88 ± 1.88	0.012	
CD90+CD3+	1	16.56 ± 2.26	15.60 ± 1.56	0.738	
	2	3.40 ± 0.81	2.76 ± 0.61	2.214	2.980
	3	15.04 ± 3.06	14.80 ± 1.80	0.028	
NK1.1	1	2.04 ± 0.21	1.84 ± 0.27	1.952	
	2	1.54 ± 0.43	1.22 ± 0.25	2.279	5.801
	3	4.54 ± 1.62	3.50 ± 1.16	1.570	
Marrow					
CD45	1	15.40 ± 1.67	14.60 ± 3.13	0.312	
	2				2.372
	3	14.60 ± 3.72	11.60 ± 3.29	2.060	
IgM+	1	17.00 ± 0.71	16.20 ± 1.48	1.382	
	2				3.272
	3	10.40 ± 4.28	13.00 ± 1.41	1.890	
IgM+IgD−	1	9.40 ± 2.30	9.00 ± 1.00	0.157	
	2				0.491
	3	9.20 ± 3.56	10.20 ± 2.39	0.334	
IgM+IgD+	1	7.60 ± 0.89	7.40 ± 2.07	0.049	
	2				1.445
	3	4.60 ± 2.07	5.80 ± 1.30	1.396	
Thymus					
CD90+CD3+	1	14.60 ± 3.98	15.60 ± 3.72	0.209	
	2	13.60 ± 2.70	13.40 ± 3.65	0.012	0.250
	3	11.20 ± 3.77	11.60 ± 4.51	0.029	
CD4+CD8−	1	16.60 ± 4.51	15.20 ± 1.64	0.519	
	2	17.20 ± 3.03	24.20 ± 6.87	4.336	5.759
	3	8.60 ± 2.19	9.60 ± 1.34	0.904	
CD4−CD8+	1	3.20 ± 1.10	3.60 ± 0.55	0.645	
	2	2.60 ± 0.89	2.60 ± 0.89	0	0.892
	3	1.60 ± 0.89	1.80 ± 0.45	0.247	
CD4+CD8+	1	74.00 ± 6.16	74.80 ± 2.28	0.092	
	2	76.00 ± 2.92	68.40 ± 8.99	3.394	4.615
	3	86.80 ± 3.90	84.80 ± 2.39	1.129	
Function					
SI (ratio of cell numbers)	1	1.72 ± 0.38	1.52 ± 0.55	0.546	
	2	2.17 ± 0.63	2.05 ± 0.49	0.141	1.887
	3	3.89 ± 3.47	2.32 ± 0.18	1.200	
CTLa, %	1	44.40 ± 7.20	38.00 ± 7.14	2.222	
	2				10.232*
	3	40.20 ± 4.97	25.60 ± 9.15	8.010	
NKa, %	1	22.20 ± 2.28	20.20 ± 2.95	1.655	
	2	28.80 ± 3.90	28.20 ± 8.01	0.028	1.705
	3	31.60 ± 6.31	31.00 ± 7.91	0.022	

Values are means ± SD. *$P < 0.05$.

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

	Sham		Electromagnetic Field					
	21 days	75 days	1 day	5 days	10 days	21 days	49 days	105 days
Cellularity, no. of cells $\times 10^7$								
Spleen						17.85		
Thymus							8.34	10.36
Bone Marrow								
Distribution, %								
Spleen								
CD45								11.34
IgM+							8.23	
IgM+IgD−						12.67		
IgM+IgD+		8.35				8.68		9.86
CD90+CD3+				9.41				
NK1.1							9.28	
Marrow								
CD45								
IgM+						12.71		
IgM+IgD−								
IgM+IgD+						16.82		11.46
Thymus								
CD90+CD3+							7.86	
CD4+CD8−								
CD4−CD8+		8.86				12.97		
CD4+CD8+			8.60		13.42			
Function								
SI (ratio of cell numbers)								7.83
CTLa, %	12.50		7.99	8.51	8.50			
Nka, %			9.98				8.14	13.97

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

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 ($P \approx 0.08$) and for 1 and 10 days ($P \approx 0.26$). 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

Fig. 1. Cumulative frequency of immune parameters (out of 20) affected by electromagnetic field exposure to 1 G, 60 Hz for 1–105 days as a function of the magnitude of the test statistic. Regions beyond the dotted line indicate $P < 0.05$ ($L > 7.83$).

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).

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

Replicate No.	x_k	y_k	l	L
1	0.698 ± 0.350	0.524 ± 0.251	2.548	7.945*
2	0.438 ± 0.339	0.685 ± 0.217	5.391	
3	0.569 ± 0.295	0.577 ± 0.214	0.006	

Values are means $\pm$ 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

1. **Adair RK.** Constraints on biological effects of weak extremely low-frequency electromagnetic fields. *Physiol Rev* 43: 1039–1048, 1991.

2. **Adair RK, Boembergen N, Bodansky D, Cormack A, Gilbert W, Glashow SL, Hafemeister D, Kobrak H, Merritt JH, Moulder JE, Park RL, Pound RV, Seaborg GT, Yalow R, and Wilson R.** *Amicus Curiae Brief, San Diego Gas & Electric Company v Marie Covalt et al.*, Supreme Court of California, Sacramento, CA, 1996.

3. **Albert EN, Slaby F, Roche J, and Loftus J.** Effect of amplitude-modulated 147 MHz radio frequency radiation on calcium ion efflux from avian brain tissue. *Radiat Res* 109: 19–27, 1987.

4. **American Physical Society.** *Power Line Fields and Public Health* (statement by the Council of the American Physical Society, Washington, DC), 1995.

5. **Anderson TW.** *An Introduction to Multivariate Statistical Analysis*. New York: Wiley, 1984.

6. **Bawin SM and Adey WR.** Sensitivity of calcium binding in cerebral tissue to weak environmental electric fields oscillating at low frequency. *Proc Natl Acad Sci USA* 73: 1999–2003, 1976.

7. **Bezrukov SM and Vodyanoy I.** Signal transduction across alamethicin ion channels in the presence of noise. *Biophys J* 73: 2456–2464, 1997.

8. **Blanchard JP and Blackman CF.** Clarification and application of an ion paramagnetic resonance model for magnetic field interactions with biological systems. *Bioelectromagnetics* 15: 217–238, 1994.

9. **Delgado JM, Leal J, Monteagudo J, and Gracia MG.** Embryological changes induced by weak, extremely low-frequency electromagnetic fields. *J Anat* 134: 533–551, 1982.

10. **Goodman R, Wei L, Xu JC, and Henderson A.** Exposure of human cells to low-frequency electromagnetic fields results in quantitative changes in transcripts. *Biochem Biophys Acta* 1009: 216–220, 1989.

11. **Jackson JD.** Are the stray 60 Hz electromagnetic fields associated with the distribution and uses of electrical power a significant cause of cancer? *Proc Natl Acad Sci USA* 89: 3508–3510, 1992.

12. **Liboff AR.** Cyclotron resonance in membrane transport. In: *Interaction between Electromagnetic Fields and Cells*, edited by Chiabrera A, Nicolini C, and Schwan HP. New York: Plenum, 1985, p. 281–296.

13. **Lorenz EN.** Deterministic nonperiodic flow. *J Atmos Sci* 20: 130–141, 1963.

14. **Maffeo S, Miller MW, and Carstensen EL.** Lack of effect of weak low frequency electromagnetic fields on chick embryogenesis. *J Anat* 139: 613–618, 1984.

15. **Margonato B, Veicsteinas A, Conti R, Nicolini P, and Cerretelli P.** Biologic effects of prolonged exposure to ELF electromagnetic fields in rats. I. 50-Hz electric fields. Bioelectromagnetics 14: 479–493, 1993.

16. **Marino AA.** Different outcomes in biological experiments involving weak EMFs: is chaos a possible explanation? *Am J Physiol Regulatory Integrative Comp Physiol* 268: R1013–R1018, 1995.

17. **Marino AA.** Electromagnetic fields, cancer, and the theory of neuroendocrine-related promotion. *Bioelectrochem Bioenerg* 29: 255–276, 1993.

18. **Marino AA and Becker RO.** High-voltage lines: hazard at a distance. *Environment* 20: 6–15, 40, 1978.

19. **Marino AA, Becker RO, and Ullrich B.** The effect of continuous exposure to low frequency electric fields on three generations of mice: a pilot study. *Experientia* 32: 565, 1976.

20. **Marino AA, Reichmanis M, Becker RO, Ullrich B, and Cullen JM.** Power frequency electric field induces biological changes in successive generations of mice. *Experientia* 36: 309–311, 1980.

21. **Merritt R, Purcell C, and Stroink G.** Uniform magnetic field produced by 3, 4, and 5 square coils. *Rev Sci Instrum* 54: 879–882, 1983.

22. **Murthy KK, Rogers WR, and Smith HD.** Initial studies of the effects of combined 60-Hz electric and magnetic field exposure on the immune system of non-human primates. *Bioelectromagnetics* 3: 93–102, 1995.

23. **Perry FS, Reichmanis M, Marino A, and Becker R.** Environmental power-frequency magnetic fields and suicide. *Health Phys* 41: 267–277, 1981.

24. **Phillips RD, Anderson LB, and Kaune WT.** *Biological Effects of High-Strength Electric Fields on Small Laboratory Animals*. Richland, WA: Pacific Northwest Laboratories, 1979.

25. **Ruelle D.** *Chance and Chaos*. Princeton, NJ: Princeton University Press, 1991.

26. **Saffer JD and Thurston SJ.** Short exposures to 60 Hz magnetic fields do not alter MYC expression in HL60 or Daudi cells. *Radiat Res* 144: 18–25, 1995.

27. **Savitz DA and Loomis DP.** Magnetic field exposure in relation to leukemia and brain cancer mortality among electric utility workers. *Am J Epidemiol* 141: 123–134, 1995.

28. **Skinner JE, Molnar M, Vybiral T, and Mitra M.** Application of chaos theory to biology and medicine. *Integr Physiol Behav Sci* 27: 39–53, 1992.

29. **Sobel E, Davanipour Z, Sulkava R, Erkinjuntti T, Wikstrom J, Henderson VW, Buckwalter G, Bowman JD, and Lee P-J.** Occupations with exposure to electromagnetic fields: a possible risk factor for Alzheimer's disease. *Am J Epidemiol* 142: 515–524, 1995.

30. **Stevens CF, Savitz DA, Anderson LE, Driscoll DA, Gage FH, Garwin RL, Jelinski LW, Kelman BJ, Luben RA, Reiter RJ, Slovic P, Stolwijk JAJ, Stuchly MA, Wartenberg D, Waugh JS, and Williams JR.** *Possible Health Effects of Exposure to Residential Electric and Magnetic Fields*. Washington, DC: National Academy Press, 1997.

31. **Weaver JC and Astumian RD.** The response of living cells to very weak electric fields: the thermal noise limit. *Science* 247: 459–462, 1990.

32. **Wertheimer N and Leeper E.** Electric wiring configuration and childhood cancer. *Am J Epidemiol* 109: 273, 1979.

33. **Wilson BW, Caputa K, Stuchly MA, Saffer JD, Davis KC, Washam CE, Washam LG, Washam GR, and Wilson MA.** Design and fabrication of well confined uniform magnetic field exposure systems. *Bioelectromagnetics* 15: 563–577, 1994.

34. **Wolcott RM, Jennings SJ, and Chervenak R.** In utero exposure to ethanol affects postnatal development of T- and B-lymphocytes, but not natural killer cells. *Alcohol Clin Exp Res* 19: 170–176, 1995.