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Nonlinear Dynamical Law Governs Magnetic Field Induced Changes in Lymphoid Phenotype

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The results of many different types of animal and human studies dealing with the biological effects of exposure to low-frequency electromagnetic fields (EMFs) have consistently been both positive and negative. We addressed the question of why this pattern had occurred so commonly in biological studies involving exposure to EMFs, and hypothesized that it stemmed from the prevalent use of a linear model to characterize what are inherently nonlinear input-output relationships. The hypothesis was tested by analyzing biological data using a novel statistical procedure that could be adjusted to detect either nonlinear or linear effects. The reliability of the procedure was established using positive and negative controls, and by comparison with the results obtained from sampling a known nonlinear system. In 4 independent experiments, male and female mice were exposed continuously to 0.1 or 0.5 mT, 60 Hz, for 175 days and the effect on 20 immune parameters was measured using flow cytometry and functional assays. In each experiment, EMF exposure resulted in statistically significant changes in lymphoid phenotype when and only when the response of the animals to the fields was analyzed as if it were governed by nonlinear laws. Our results suggest that the pattern of inconsistency in the EMF bioeffects studies is an artifact resulting from an incorrect choice of the conceptual model for the relation between the field and the biological effect it causally determines. Bioelectromagnetics 22:529—546, 2001. © 2001 Wiley—Liss, Inc.

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INTRODUCTION

Interest in understanding the biological effects of exposure to low frequency electromagnetic fields (EMFs) has led to many different kinds of laboratory and epidemiological studies. Unfortunately the published reports have characteristically lacked regular and verifiable consistency [Bawin and Adey, 1976; Marino et al., 1976; Marino et al., 1977; Phillips et al., 1979; Delgado et al., 1982; Maffeo et al., 1984; Quinlan et al., 1985; Thomas et al., 1986; Wilson et al., 1986; Albert et al., 1987; Goodman et al., 1989; Sasser et al., 1991; Margonato et al., 1993; Murthy et al., 1995; Saffer and Thurston, 1995; Savitz and Loomis, 1995; Stern et al., 1996; Li et al., 1997]. Primarily because of this pattern of inconsistency, the public-health significance of EMFs has been discounted [American Physical Society, 1995; Stevens et al., 1997; Portier and Wolfe, 1998; Olden, 1999]. We addressed ourselves to the question of why the pattern of positive and negative reports has occurred so commonly.

In almost all EMF studies it was effectively assumed that any response of a subject to a field would be governed by a linear law and that inter-subject measurement differences were due solely to stochastic processes. In this type of generalized picture of dynamical activity (model), a deterministic response must be consistent from animal to animal. If it were the case in a laboratory experiment, for example, that an EMF produced an increase in a parameter in one animal and a decrease or no effect in a second animal, that result would violate either the assumption that the response was deterministic or the assumption that the animals were identical. Thus, in a

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linear stochastic model, lack of a consistent response in a group of identical animals entails lack of determinism. In a nonlinear model, in contrast, the absence of the kind of consistency found in linear systems cannot properly be interpreted to evidence indeterminism [Ruelle, 1991; Schuster, 1992].

Several considerations suggested to us that the relationship between an applied EMF and its associated bioeffect was generally nonlinear in nature. First, if only the negative reports [Phillips et al., 1979; Maffeo et al., 1984; Quinlan et al., 1985; Albert et al., 1987; Sasser et al., 1991; Margonato et al., 1993; Saffer and Thurston, 1995; Stern et al., 1996] were objectively correct, it would be difficult to explain the frequency of the positive reports [Bawin and Adey, 1976; Marino et al., 1976; Marino et al., 1977; Delgado et al., 1982; Thomas et al., 1986; Wilson et al., 1986; Goodman et al., 1989; Murthy et al., 1995; Savitz and Loomis, 1995; Li et al., 1997], because each of them was protected against chance at $P < 0.05$. On the other hand, under a nonlinear model, both kinds of reports could be true and consistent with the presence of determinism. Second, kT based arguments against the possibility of EMF transduction (where k is Boltzman's constant and T the temperature) depend on the assumption of a linear model [Adair, 1991]. But even an infinitesimally small input to a nonlinear system can result in macroscopic dynamical changes [Lorenz, 1963]. Thus, the applicability of a nonlinear model could explain why the kT argument is at variance with the biological data. Third, both strong [Bassett, 1985] and more physiological strength [Capanna et al., 1994] EMFs have been proven capable of eliciting identical clinical responses, suggesting a nonlinear link between the field and healing.

We previously used a novel statistical procedure capable of detecting either nonlinear or linear change in response to a stimulus. We employed the procedure to show that exposure of mice to 0.1 mT, 60 Hz, for 1-105 days caused nonlinear changes among 20 immune parameters, as determined using flow cytometry and functional assays [Marino et al., 2000]. The present study was undertaken to determine if the nonlinear approach was also successful under other conditions of exposure and whether the impact of EMFs on the immune system selectively impacted the natural immunosurveillance function of the immune system, as previously suggested [Marino, 1993].

METHODS

Experimental Design

In earlier EMF bioeffects studies it was generally assumed that any effect would be linear and unidirectional, and would occur consistently in a particular parameter. Sometimes the assumptions were explicit, but more often they were implicit in the statistical procedures utilized. In contrast, we assumed that: (1) a true deterministic response could be either an increase or a decrease, depending on the animal; and (2) for a given set of parameters, the particular parameters affected by the field could be *a priori* undeterminable. A roulette wheel exemplifies the latter idea. An input (releasing the ball) always results in an output (ball in a slot), but the particular slot is not predictable [Ford, 1983].

To characterize the immune system, we measured a total of 20 standard immune parameters in each animal (see below). We recognized that the mean of any individual parameter in a large sample of exposed mice, each exhibiting the assumed behavior, would be similar to the mean of the controls, even if the field produced a deterministic response in every exposed animal. In other words, if the underlying dynamical activity were as we hypothesized, an effect of the EMF would not be observed by comparing means in large samples because oppositely directed changes would be averaged away. A single small sample might reveal the putative effect due to incomplete averaging, but statistical tests on small samples generally lack statistical power. To overcome the latter problem, we developed a novel statistical procedure suitable for inferring the occurrence of irregular changes.

Statistical Procedure

The likelihood approach allows differences in means from replicate series of exposed and control groups to be combined to test an overall hypothesis [Anderson, 1984], 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

$$l = 2N \ln\left[1 + \frac{1}{2N-2} t^2\right] \quad (1)$$

where N is the number of animals in each group [Lockhart, 2000]. The distribution of l is approximately χ^2 with 1 degree of freedom. For k pairs, the overall values of the test statistic, L, is $L = \sum_1^k l_i$, which 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.

In preliminary studies, we evaluated the use of the log-likelihood ratio for the F test for differences in variance. We found an unacceptably high number of false positive results and ultimately determined that they arose because of the statistical properties of the variance, in particular, because the variance of sample variance is always greater than the variance of the

sample mean. Consequently, differences in variances were not incorporated into our statistical design.

Data Evaluation

The occurrence of changes in the immune system due to exposure to 60-Hz magnetic fields was assessed on the basis of whether $L > \chi^2_{k,0.05}$, with k=3 and N=5, except for one experiment (male mice exposed at 0.1 mT), where k=4. We chose a group size of 5 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 below). Controls included sham exposed mice paired with each exposed group and two experiments in which both groups were sham exposed.

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 3 replicates prior to analysis ($L > \chi^2_{1,0.05}$, with N=15 or 20, as appropriate). This procedure was equivalent to performing a t test on the combined data. L for small samples (N=5) is not precisely chi-square, but we showed that the error was immaterial (see Discussion below). The binomial theorem was used to assess whether the observed number of statistically significant L values in a particular experiment could reasonably be attributed to chance.

More than 3600 individual measurements of 20 different immune parameters were made. Of this total, about 15 measurements differed by more than 5 standard deviations from their respective means. The outliers were included in the analysis because we had no objective basis to exclude them.

MathCad (MathSoft, Cambridge, MA) was used for all computations. Percentile plots [Wilk and Gnanadesikan, 1968] were made using Statview (Abacus, Berkeley, CA) by normalizing the data within each replicate by the mean of the control group and then combining replicates.

Exposure System

Horizontal magnetic fields were produced using an arrangement of four square coils, 48 cm on a side [Merritt et al., 1983], with construction details generally as specified by Wilson et al. [Wilson et al., 1994]. The outer and inner coils in each 4 coil system, overall length 51 cm, consisted of 85 and 35 turns, respectively, of 12 gauge magnet wire. Each coil was dipped in epoxy to minimize potential effects due to vibration and wrapped with grounded metal shielding to eliminate the possibility of electric-field effects. The shield was interrupted to prevent magnetic fields due to eddy currents. Each four coil system accommodated four cages on two shelves. Four sets of four coil systems, arranged in an octapole configuration [Wilson et al., 1994] constituted an exposure unit.

The units were designed using commercial software (MF3D, ERM Inc., Pittsburgh, PA) to produce a uniform magnetic field (±5%) throughout the volume occupied by the mice (0.03 m), with a negligible fringing field. The predicted uniformity and an absence of fringing field beyond 2 m from the unit were verified by direct measurements (Bartington MAG-03, GMW, Redwood City, CA).

Four identical units were built. Two were used to produce magnetic fields, and the others were short circuited and used to house the control mice. The exposure and control units were separated by more than 5 m in a room in the institutional animal care facility that was chosen because of its low ambient 60 Hz magnetic fields and uniform geomagnetic field. The ambient 60 Hz field at the locations of the control units averaged 0.04 μT and was never higher than 0.07 μT. The earth's field was approximately 0.053 mT, at an angle of 62° below the horizon.

The exposure units were energized by power supplies consisting of an isolation transformer, autotransformer, and series capacitors. The units were operated in series resonance at 60 Hz to eliminate powerline harmonics. Fourier analysis of the coil currents showed that the strongest harmonics were 50 dB below the fundamental. The power supplies were rack mounted in solid copper boxes to minimize the magnetic fields created by eddy currents caused by the transformers.

The exposure room was continuously maintained under temperature and humidity control with an unvarying light-dark cycle (12/12). Room air was replaced 15 times/hour with fresh air. Sentinel mice were screened for the presence of hepatitis and Sendai virus. 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; the parameters remained at their design levels throughout the study (<5% variation).

Animals

Male and female C57BL/6 mice (Jackson Laboratories, Bar Harbor, MN), 6 weeks old at arrival, were rested a minimum of 2 weeks before use. The mice were housed in groups of four in a nonmetallic environment consisting of a polystyrene cage (30 x 22 x 16 cm) and microbarrier filter top. The water bottle (glass sipper) was placed inside the cage to minimize differences in electrical potential between the mice and the

water. Food pellets were placed in an accessible area of the bottle holder. The animal cages sat on plastic shelves whose indentations automatically centered the cages in the homogeneous portion of the magnetic field. The shelves were wall mounted to prevent vibrational coupling between the coils and the cages. After commencing an experiment, the mice were exposed continuously, except for the time needed to service the cages (about 1 h/week). To avoid potential confounding effects due to switching transients, the magnetic field was never changed during the time the mice were exposed.

In separate experiments, male and female mice were exposed to 0.1 mT and 0.5 mT, 60 Hz, for 175 days. The field strengths were chosen because they are near the largest that would ordinarily be encountered in the environment. The frequency was chosen to be that of the North American power system. 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. In each experiment, three replicates were evaluated, each consisting of five exposed and five control mice. The 10 mice in a particular pair were sacrificed (cervical dislocation) on the same morning, and the minimum time between sacrifice of any two pairs was 1 week.

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 phosphate buffered saline (PBS). The cells were counted (Z1, Coulter, Hialeah, FL) and then resuspended at 10^7 cell/ml in staining buffer (PBS, 2% fetal bovine serum, 1 g/liter sodium azide). 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 of the B6 mouse, 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-CD45 for antigens on B cells. Antibodies were purified from hybridomas (ATCC, 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 cultures (stimulation index). The lytic activity of spleen NK cells was enhanced by culturing spleen cells for 18 h in medium containing IL-2 (800 units/ml) [Wolcott et al., 1995].

^{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, and the mean was used in the analysis. 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 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 NK cell assay was included because earlier work suggested that impairment of the NK cell lymphoid subpopulation was responsible for health consequences associated with EMF exposure [Marino, 1993]. However, no *a priori* assumptions were made regarding the relative importance of any immune measure during the statistical evaluation.

RESULTS

Twenty immune parameters were measured in each of five male mice exposed to 0.1 mT for 175 days and in each of five sham exposed control mice, and the mean ± SD for each parameter was determined. Using Equation (1), l was calculated for each of the 20 comparisons between the two groups. The entire procedure was performed three additional times, totaling four 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 four degrees of freedom and found 10 statistically significant comparisons (Table 1).

TABLE 1. Immune Parameters (mean ± SD) in Male Mice Exposed in Four Replicates to 0.1 mT, 60 Hz, for 175 Days.

	Replicate	*E*	*C*	*l*	*L*
Cellularity (no. of cells x10^7):					
Spleen	1	12.02 ± 1.89	11.88 ± 1.20	0.024	
	2	12.14 ± 1.44	11.92 ± 1.85	0.055	1.091
	3	9.74 ± 0.99	9.26 ± 2.43	0.207	
	4	13.30 ± 0.71	12.85 ± 0.88	0.805	
Thymus	1	4.70 1.01	4.02 ± 0.30	2.328	
	2	4.68 ± 0.78	4.46 ± 2.14	0.058	
	3	2.52 ± 0.89	1.92 ± 1.04	1.136	4.278
	4	4.60 ± 0.83	4.18 ± 0.73	0.756	
Bone Marrow.....	1	3.36 ± 0.15	3.42 ± 0.66	0.049	
	2	3.92 ± 0.75	3.52 ± 0.39	1.303	
	3	3.64 ± 1.15	4.32 ± 1.82	0.603	2.123
	4	3.90 ± 0.93	3.65 ± 1.05	0.168	
Distribution (%):					
Spleen:					
CD45.....	1	3.00 ± 0.71	2.40 ± 0.55	2.478	
	2	2.60 ± 0.89	2.40 ± 0.55	0.224	
	3	2.60 ± 0.55	3.00 ± 1.00	0.740	4.326
	4	2.00 ± 0.00	3.25 ± 0.50	0.884	
IgM+	1	66.00 ± 2.34	63.00 ± 2.00	4.649	
	2	63.40 ± 2.41	63.40 ± 1.14	0.000	
	3	60.00 ± 3.54	61.60 ± 2.19	0.000	5.226
	4	59.50 ± 4.04	57.75 ± 3.30	0.577	
IgM+IgD-.....	1	9.40 ± 1.14	10.20 ± 1.30	1.252	
	2	11.40 ± 0.89	10.40 ± 0.55	4.499	
	3	10.80 ± 1.10	10.80 ± 0.84	0.000	5.751
	4	15.75 ± 1.26	15.75 ± 2.63	0.000	
IgM+IgD+.....	1	57.80 ± 1.64	53.40 ± 3.21	6.580	
	2	54.00 ± 3.08	53.20 ± 2.68	0.237	
	3	48.60 ± 3.58	50.60 ± 2.51	1.229	*11.200
	4	49.75 ± 3.69	44.75 ± 4.57	3.154	
CD90+CD3+.....	1	16.60 ± 0.89	18.80 ± 2.05	4.731	
	2	15.60 ± 0.89	14.00 ± 0.71	8.024	
	3	15.00 ± 1.41	14.60 ± 3.58	0.067	*13.084
	4	13.25 ± 0.50	13.50 ± 1.00	0.262	
NK1.1.....	1	2.42 ± 0.27	2.66 ± 0.36	1.655	
	2	2.46 ± 0.25	2.50 ± 0.12	0.127	
	3	2.30 ± 0.10	2.38 ± 0.16	1.026	3.292
	4	2.75 ± 0.25	2.65 ± 0.21	0.484	
Marrow:					
CD45.....	1	7.60 ± 1.52	9.40 ± 1.82	3.087	
	2	9.40 ± 1.14	7.20 ± 1.79	5.142	
	3	5.60 ± 2.61	6.60 ± 1.82	0.601	9.002
	4	9.50 ± 2.08	9.00 ± 1.83	0.172	
IgM+	1	11.00 ± 1.41	12.20 ± 3.27	0.685	
	2	11.00 ± 1.00	10.40 ± 1.14	0.934	
	3	9.20 ± 2.28	11.60 ± 1.67	3.714	5.333
	4	11.25 ± 1.71	11.25 ± 1.89	0.000	

(continued)

TABLE 1. *(Continued)*

	Replicate	*E*	*C*	*l*	*L*
IgM+IgD-.....	1	7.40 ± 4.28	7.40 ± 1.52	0.000	
	2	6.40 ± 2.61	5.20 ± 1.64	0.906	
	3	5.00 ± 2.83	5.40 ± 2.70	0.065	2.339
	4	7.25 ± 2.22	6.00 ± 0.82	1.368	
IgM+IgD+.....	1	7.60 ± 1.14	8.20 ± 2.68	0.261	
	2	6.40 ± 0.55	7.20 ± 0.84	3.365	
	3	6.60 ± 0.89	8.00 ± 1.00	5.190	*9.783
	4	6.25 ± 0.96	7.00 ± 1.41	0.967	
Thymus:					
CD90+CD3+.....	1	10.80 ± 1.64	13.40 ± 3.29	2.722	
	2	14.60 ± 1.34	13.80 ± 1.10	1.252	
	3	12.40 ± 2.07	19.40 ± 4.98	7.192	*13.148
	4	16.25 ± 3.78	13.50 ± 1.92	1.982	
CD4+CD8-.....	1	7.80 ± 0.45	9.80 ± 1.64	6.216	
	2	10.00 ± 1.87	11.80 ± 1.30	3.289	
	3	12.80 ± 1.48	14.80 ± 1.92	3.532	*28.356
	4	9.25 ± 0.50	11.50 ± 0.58	15.319	
CD4-CD8+.....	1	1.14 ± 0.18	1.64 ± 0.41	5.752	
	2	2.16 ± 0.55	1.88 ± 0.31	1.156	
	3	2.38 ± 0.80	3.44 ± 1.49	2.188	*10.180
	4	2.30 ± 0.44	2.00 ± 0.47	1.084	
CD4+CD8+.....	1	87.40 ± 0.55	84.80 ± 1.79	7.918	
	2	83.80 ± 3.11	82.40 ± 1.52	0.973	
	3	80.60 ± 2.97	76.20 ± 5.26	2.865	*11.756
	4	81.75 ± 0.96	81.75 ± 1.26	0.000	
Function:					
SI (ratio of cell numbers)	1	2.08 ± 0.61	1.44 ± 0.07	5.176	
	2	1.64 ± 0.12	2.04 ± 0.73	1.660	
	3	1.19 ± 0.37	1.56 ± 0.27	3.490	*10.932
	4	2.32 ± 0.51	2.10 ± 0.38	0.606	
CTLa (%).....	1	35.40 ± 8.17	42.80 ± 6.69	2.677	
	2	59.20 ± 4.76	66.40 ± 2.79	7.238	
	3	94.60 ± 4.34	93.80 ± 4.21	0.109	*13.391
	4	42.75 ± 11.30	54.50 ± 6.95	3.367	
NKa (%).....	1	20.20 ± 3.56	20.00 ± 2.00	0.015	
	2	12.20 ± 3.11	13.20 ± 3.70	0.263	*11.734
	3	15.00 ± 1.83	9.25 ± 1.89	11.456	
	4	—	—	—	

Note: SI, Stimulation index; CTLa, Cytotoxic T lymphocyte assay (E:T, 13:1); NKa, Natural killer cell cytotoxic assay (E:T, 25:1); Dashes indicate the absence of data due to technical errors.

*$P<0.05$

The experiment was performed again using female mice, but employing a total of only three replicates and nine significant comparisons were observed (Table 2). The experiments using male and female mice were both performed again using a field of 0.5 mT (a total of three replicates in each experiment), with the result that three and five significant changes were found in the male and female mice, respectively (Tables 3 and 4).

To explore the possibility that the relatively large numbers of significant differences were somehow a by-product of our novel statistical procedure, the experiment was performed two additional times with no field applied to the putatively exposed group in either sham study. The sham exposure lasted 21 days in one case and 75 days in the other. The durations were chosen for convenience; the numerical values were unimportant because the purpose of the

TABLE 2. Immune Parameters (mean ± SD) in Female Mice Exposed in Three Replicates to 0.1 mT, 60 Hz, for 175 Days

	Replicate	*E*	*C*	*l*	*L*
Cellularity (no. of cells x10^7):					
Spleen	1	9.56 ± 0.77	9.08 ± 1.67	0.416	
	2	9.32 ± 1.52	9.88 ± 0.99	0.581	1.140
	3	11.50 ± 1.63	11.88 ± 1.89	0.143	
Thymus	1	3.22 ± 0.77	3.98 ± 0.63	3.112	
	2	3.72 ± 1.05	2.76 ± 0.58	3.356	*7.998
	3	3.30 ± 0.65	2.64 ± 1.11	1.530	
Bone Marrow.....	1	2.98 ± 0.16	3.42 ± 0.69	2.172	
	2	3.28 ± 0.73	3.06 ± 0.54	0.364	*9.593
	3	3.36 ± 0.38	4.42 ± 0.74	7.057	
Distribution (%):					
Spleen:					
CD45.....	1	2.12 ± 0.22	2.46 ± 0.78	1.039	
	2	2.72 ± 0.55	3.68 ± 0.48	7.319	*11.584
	3	5.08 ± 0.47	6.04 ± 1.02	3.226	
IgM+	1	53.70 ± 4.62	48.56 ± 3.31	4.128	
	2	59.54 ± 1.74	56.42 ± 1.95	6.364	*11.961
	3	58.15 ± 4.02	54.66 ± 5.10	1.469	
IgM+IgD-.....	1	18.70 ± 1.08	28.42 ± 1.58	28.419	
	2	17.38 ± 1.36	16.18 ± 1.49	1.998	*30.535
	3	22.38 ± 2.13	21.90 ± 2.47	0.118	
IgM+IgD+.....	1	46.08 ± 1.95	37.16 ± 3.70	13.475	
	2	45.64 ± 1.99	44.36 ± 1.72	0.863	*16.029
	3	42.10 ± 3.09	39.26 ± 3.81	1.691	
CD90+CD3+.....	1	10.50 ± 2.30	7.12 ± 1.30	7.040	
	2	5.88 ± 1.55	5.80 ± 0.97	0.012	*11.400
	3	10.28 ± 0.92	8.56 ± 1.41	4.348	
NK1.1.....	1	1.92 ± 0.88	1.16 ± 0.50	3.003	
	2	2.26 ± 0.44	2.02 ± 0.13	1.582	4.973
	3	0.60 ± 0.29	0.72 ± 0.34	0.388	
Marrow:					
CD45.....	1	6.28 ± 1.94	6.16 ± 1.11	0.018	
	2	8.78 ± 0.86	8.32 ± 1.59	0.395	3.135
	3	11.06 ± 1.93	8.50 ± 3.06	2.722	
IgM+	1	11.82 ± 1.81	12.10 ± 2.46	0.052	
	2	10.96 ± 2.08	12.10 ± 1.28	1.280	5.831
	3	12.98 ± 1.15	9.48 ± 3.49	4.499	
IgM+IgD-.....	1	10.02 ± 3.21	13.20 ± 4.90	1.691	
	2	7.58 ± 1.04	7.34 ± 1.65	0.094	5.211
	3	12.98 ± 2.14	10.42 ± 2.34	3.426	
IgM+IgD+.....	1	6.94 ± 1.30	6.96 ± 1.24	0.001	
	2	6.04 ± 1.99	7.96 ± 1.39	3.295	5.810
	3	6.90 ± 1.90	4.66 ± 2.71	2.514	
Thymus:					
CD90+CD3+.....	1	6.48 ± 0.70	7.26 ± 1.51	1.291	
	2	7.78 ± 1.56	6.12 ± 0.47	5.031	*8.172
	3	11.28 ± 2.36	19.20 ± 13.69	1.850	

(continued)

TABLE 2. *(Continued)*

	Replicate	*E*	*C*	*l*	*L*
CD4+CD8-.....	1	19.38 ± 3.30	23.68 ± 6.48	1.976	
	2	8.10 ± 1.55	8.88 ± 2.60	0.408	7.266
	3	35.72 ± 8.65	49.36 ± 10.48	4.882	
CD4-CD8+.....	1	0.84 ± 0.43	1.36 ± 0.49	3.375	
	2	1.42 ± 0.30	1.54 ± 0.38	0.368	4.371
	3	1.68 ±0.65	2.94 ± 3.86	0.628	
CD4+CD8+.....	1	71.42 ± 2.75	66.36 ± 7.21	2.379	
	2	84.72 ± 2.18	84.24 ± 3.37	0.089	7.672
	3	55.36 ± 8.85	35.00 ± 17.35	5.204	
Function:					
SI (ratio of cell numbers)	1	1.53 ± 0.48	1.12 ± 0.64	1.498	
	2	1.22 ± 0.10	1.17 ± 0.10	0.853	4.153
	3	3.36 ± 1.39	2.35 ± 1.12	1.802	
CTLa (%).....	1	66.20 ± 5.36	67.00 ± 9.85	0.032	
	2	29.40 ± 5.32	19.60 ± 4.93	7.612	7.648
	3	68.60 ± 7.40	68.80 ± 3.96	0.004	
NKa (%).....	1	14.60 ± 1.14	16.60 ± 1.52	5.273	
	2	31.60 ± 3.85	21.60 ± 2.30	14.134	*19.539
	3	24.00 ± 3.94	23.20 ± 3.83	0.132	

Note: SI, Stimulation index. CTLa, cytotoxic T lymphocyte assay (E:T, 13:1) and NKa, Natural killer cell cytotoxic assay (E:T, 25:1).

*$P<0.05$

experiments was to ascertain the frequency of false positive results produced by our statistical procedure. We found one significant difference in each experiment (data not shown, but see Table 4 in [Marino et al., 2000]. 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 Figure 1.

To examine the ability of linear statistical analysis to detect the deterministic effect of the field, in each experiment, *L* was computed directly from the 15 exposed and 15 control mice (20 exposed and 20 control mice for the males exposed at 0.1 mT) and evaluated based on the χ^2 distribution with one degree of freedom. The procedure produced no significant differences in any instances where effects were noted initially (Table 5).

We performed an additional check on the reliability of the replicate procedure for distinguishing between nonlinear and linear behavior. The parameters in the Lorenz system of nonlinear equations [Lorenz, 1963] were chosen so that the system was in the chaotic mode, and the evolution of one of the variables (temperature) was calculated over 300 s for initial values of 24 and 26°C. Three replicates (N=5 in each) were formed from each time series by random sampling and the 2 series were compared to determine whether $L > \chi^2_{3,0.05}$. A typical result is shown in Table 6. The procedure correctly recognized that the two populations actually differed (L = 9.01, $P < 0.05$). When the 15 individual samples were combined to compute whether $L > \chi^2_{1,0.05}$, no effect was found (L = 2.45, $P > 0.05$).

To assess whether the field selectively impacted animals in different portions of the distribution of measurements, percentile plots were made for parameters that were significantly affected by the field in both genders (Table 5). At 0.1 and 0.5 mT, the functional activity of the NK cells in the lower tail of the distribution was reduced as a consequence of the exposure and the activity of cells in the upper tail was increased (Fig. 2). Spleen CD90+CD3+ and IgM+IgD+ cells were significantly affected by field exposure at 0.1 mT, but not at 0.5 mT (Fig. 3). In both cases the effect occurred primarily at the upper portions of the distribution. We found comparable results for the percentile plots of thymus CD90+CD3+ cells (data not shown). The distribution of spleen cells was unaffected by exposure at 0.1 mT but was altered at 0.5 mT, particularly for the animals in the upper part of the distribution (Figure 4).

DISCUSSION

Observation of Real Change

The small sample replicate-structure procedure that we devised based on the log-likelihood ratio statistic for the *t* test

TABLE 3. Immune Parameters (mean – SD) in Male Mice Exposed in Three Replicates to 0.5˚mT, 60˚Hz, for 175 Days

	Replicate	*E*	*C*	*l*	*L*
Cellularity (no. of cells x10^7):					
Spleen	1	14.98 ± 2.40	11.54 ± 2.18	5.329	
	2	12.92 ± 2.42	9.92 ± 1.11	4.958	*10.526
	3	14.02 ± 3.39	13.18 ± 2.08	0.239	
Thymus	1	1.98 ± 1.79	3.72 ± 1.32	3.238	
	2	5.76 ± 1.19	4.72 ± 1.02	2.152	
	3	3.18 ± 1.00	3.52 ± 0.95	0.334	5.724
Bone Marrow.....	1	4.88 ± 1.21	5.58 ± 0.54	1.604	
	2	4.30 ± 0.61	4.85 ± 0.61	2.063	3.963
	3	3.80 ± 0.55	3.95 ± 0.31	0.296	
Distribution (%):					
Spleen:					
CD45.....	1	2.00 ± 0.00	2.00 ± 0.00	0.000	
	2	2.60 ± 0.55	3.25 ± 0.50	3.538	3.538
	3	2.00 ± 0.00	2.00 ± 0.00	0.000	
IgM+	1	59.20 ± 2.95	60.20 ± 3.03	0.344	
	2	62.00 ± 2.34	60.50 ± 2.89	0.910	3.998
	3	61.00 ± 1.83	63.25 ± 2.22	2.744	
IgM+IgD-.....	1	13.00 ± 1.58	12.80 ± 2.68	0.026	
	2	12.20 ± 1.48	11.75 ± 1.26	0.294	2.211
	3	9.75 ± 0.50	10.75 ± 1.50	1.891	
IgM+IgD+.....	1	50.40 ± 3.36	51.20 ± 2.17	0.247	
	2	49.20 ± 2.59	47.00 ± 3.56	1.382	5.168
	3	47.75 ± 2.06	51.75 ± 3.86	3.539	
CD90+CD3+.....	1	16.60 ± 3.85	16.80 ± 1.64	0.014	
	2	15.20 ± 2.49	12.78 ± 1.71	3.012	3.880
	3	15.25 ± 2.75	16.50 ± 1.29	0.854	
NK1.1.....	1	3.48 ± 0.43	3.12 ± 0.55	1.547	
	2	2.64 ± 0.35	2.32 ± 0.39	1.898	*12.104
	3	3.05 ± 0.21	2.50 ± 0.24	8.659	
Marrow:					
CD45.....	1	4.80 ± 3.27	5.40 ± 3.13	0.109	
	2	10.00 ± 2.12	8.25 ± 1.26	2.355	3.318
	3	5.50 ± 2.08	6.75 ± 2.22	0.854	
IgM+	1	10.00 ± 5.24	10.40 ± 2.79	0.028	
	2	12.80 ± 1.64	12.25 ± 1.26	0.381	3.003
	3	7.75 ± 4.57	11.75 ± 2.63	2.594	
IgM+IgD-.....	1	7.00 ± 3.74	5.60 ± 1.95	0.666	
	2	8.60 ± 3.85	6.25 ± 3.40	1.104	4.338
	3	5.00 ± 1.16	7.25 ± 2.75	2.568	
IgM+IgD+.....	1	6.40 ± 2.70	7.40 ± 1.34	0.664	
	2	8.60 ± 1.34	8.50 ± 0.56	0.024	3.160
	3	5.50 ± 3.51	8.25 ± 1.26	2.472	
Thymus:					
CD90+CD3+.....	1	22.00 ± 15.62	8.60 ± 4.45	3.545	
	2	14.00 ± 2.65	15.25 ± 3.30	0.501	5.076
	3	11.00 ± 1.83	12.25 ± 2.06	1.030	

(continued)

TABLE 3. *(Continued)*

CD4+CD8-.....	1	17.40 ± 10.11	11.80 ± 0.84	1.742	
	2	9.40 ± 1.52	11.00 ± 1.83	2.335	4.091
	3	11.50 ± 4.36	11.25 ± 2.22	0.014	
CD4-CD8+.....	1	4.94 ± 3.86	1.08 ± 0.53	4.793	
	2	1.62 ± 0.72	1.68 ± 0.56	0.020	5.154
	3	1.80 ± 0.16	1.70 ± 0.36	0.341	
CD4+CD8+.....	1	66.00 ± 19.85	81.00 ± 0.71	3.050	
	2	82.20 ± 3.19	81.00 ± 2.16	0.510	3.560
	3	83.00 ± 4.83	83.00 ± 2.31	0.000	
Function:					
SI (ratio of cell numbers)	1	1.16 ± 0.12	1.10 ± 0.11	0.904	
	2	1.17 ± 0.28	1.12 ± 0.09	0.154	1.169
	3	1.24 ± 0.29	1.29 ± 0.16	0.111	
CTLa (%).....	1	65.80 ± 7.86	73.80 ± 4.92	3.823	
	2	55.20 ± 4.82	53.00 ± 1.02	0.235	6.410
	3	86.50 ± 11.15	77.75 ± 4.99	2.352	
NKa (%).....	1	14.00 ± 1.58	16.40 ± 0.89	7.375	
	2	41.20 ± 2.38	31.25 ± 2.99	15.244	*25.673
	3	12.25 ± 1.71	16.50 ± 4.80	3.054	

Note: SI, Stimulation index. CTLa, Cytotoxic T lymphocyte assay (E:T, 13:1) and NKa, Natural killer cell cytotoxic assay (E:T, 25:1).

*$P<0.05$

was capable of detecting any form of change induced by the field, whether or not it could be explained by linear theory. In this regard, our study differed from previous EMF bioeffects studies, most of which employed statistical designs geared to detect linear change. Chance alone produced only one significant comparison in 20 tests in each of the two sham experiments (Table 5, Fig. 1), which was the expected result. The increased frequency of significant differences observed in the male mice exposed to 0.5 mT (Table 5, Fig. 1) could conceivably also be attributed to chance, because there was a small possibility ($P = 0.08$, binomial theorem) that the differences arose from stochastic processes unrelated to the presence of the field. For the other three experiments, however, it is highly implausible that the observed frequencies of statistically significant differences (Table 5, Fig. 1) occurred by chance.

It might be argued that the large number of significant differences observed in the experiments were due to the use of the χ^2 distribution, because L for small samples (N=5) is not precisely χ^2. However, 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. Further, as mentioned, the overall reliability of the L procedure was directly verified in each of the two sham studies. We conclude, therefore, that 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.

Nature of Change

Several considerations indicated that the deterministic effect of the field on the immune system was nonlinear in nature. First, the relative value of the means of corresponding exposed and control groups typically varied from replicate to replicate (Tables 1-4). To establish the fundamental role of this variation in revealing the kind of 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 our research (averaging away real effects). This was accomplished by computing L directly from the 15 exposed and 15 control mice, thereby neglecting that the replicates were not performed at the same time, and evaluating for statistical significance based on the chi-square distribution with one degree of freedom. This procedure, which is statistically equivalent to an unpaired t test, produced no significant effects in any of the instances in which effects were noted initially (Fig. 1).

Second, a particular parameter that was significantly affected after exposure to 0.1 mT was not necessarily affected following exposure to 0.5 mT (Table 5). This behavior is

TABLE 4. Immune Parameters (mean ± SD) in Female Mice Exposed in Three Replicates to 0.5 mT, 60 Hz, for 175 Days

	Replicate	*E*	*C*	*l*	*L*
Cellularity (no. of cells x10^7):					
Spleen	1	8.17 ± 0.45	10.42 ± 2.17	3.305	
	2	10.02 ± 0.98	9.50 ± 2.66	0.208	*8.285
	3	11.34 ± 0.67	9.82 ± 1.38	4.772	
Thymus	1	4.87 ± 1.87	5.85 ± 0.85	1.106	
	2	3.80 ± 1.81	3.40 ± 0.54	0.277	1.735
	3	3.60 ± 0.64	3.36 ± 0.77	0.352	
Bone Marrow.....	1	3.47 ± 0.23	3.42 ± 0.25	0.070	
	2	1.94 ± 0.34	2.54 ± 0.39	6.044	6.551
	3	3.52 ± 0.54	3.32 ± 0.52	0.437	
Distribution (%):					
Spleen:					
CD45.....	1	1.00 ± 1.00	1.75 ± 0.50	2.104	
	2	2.60 ± 0.55	2.80 ± 0.84	0.247	3.405
	3	2.20 ± 0.45	1.80 ± 0.84	1.054	
IgM+	1	42.00 ± 8.00	51.75 ± 4.79	4.223	
	2	58.00 ± 3.08	59.00 ± 3.54	0.280	6.839
	3	61.20 ± 3.03	63.40 ± 1.52	2.336	
IgM+IgD-.....	1	20.67 ± 2.52	22.25 ± 3.78	0.523	
	2	20.60 ± 1.14	21.40 ± 3.36	0.312	2.865
	3	18.20 ± 1.48	19.40 ± 1.34	2.030	
IgM+IgD+.....	1	41.33 ± 2.31	44.50 ± 1.29	5.177	
	2	44.20 ± 1.92	45.00 ± 3.74	0.223	5.407
	3	46.80 ± 5.59	47.00 ± 2.45	0.007	
CD90+CD3+.....	1	15.67 ± 2.08	13.00 ± 2.45	2.636	
	2	16.80 ± 3.70	14.40 ± 1.52	2.030	4.666
	3	11.40 ± 2.30	11.40 ± 2.70	0.000	
NK1.1.....	1	2.30 ± 1.57	3.22 ± 0.50	1.607	
	2	2.82 ± 0.18	3.26 ± 0.62	2.531	4.221
	3	2.44 ± 0.24	2.38 ± 0.46	0.083	
Marrow:					
CD45.....	1	8.67 ± 1.11	13.55 ± 2.20	8.589	
	2	12.72 ± 2.32	15.22 ± 3.07	2.345	*11.040
	3	7.98 ± 2.09	7.50 ± 3.03	0.106	
IgM+	1	17.00 ± 2.65	18.50 ± 3.70	0.474	
	2	23.40 ± 1.14	21.60 ± 3.21	1.610	2.729
	3	17.40 ± 3.36	15.80 ± 3.56	0.645	
IgM+IgD-.....	1	14.67 ± 8.14	13.25 ± 3.50	0.141	
	2	19.00 ± 4.69	16.40 ± 5.13	0.839	1.750
	3	10.40 ± 1.14	11.20 ± 1.92	0.770	
IgM+IgD+.....	1	8.53 ± 0.46	8.10 ± 1.81	0.216	
	2	12.62 ± 2.14	11.32 ± 3.84	0.533	1.724
	3	11.98 ± 3.79	10.36 ± 1.28	0.975	
Thymus:					
CD90+CD3+.....	1	16.13 ± 1.22	16.85 ± 4.06	0.116	
	2	14.42 ± 1.96	15.40 ± 1.77	0.826	*12.260
	3	7.24 ± 0.80	4.08 ± 1.53	11.318	
CD4+CD8-.....	1	7.00 ± 1.00	7.00 ± 0.82	0.000	
	2	8.80 ± 1.10	10.00 ± 0.71	4.249	4.693
	3	10.40 ± 1.52	13.60 ± 11.76	0.444	

(continued)

TABLE 4. *(Continued)*

	Replicate	*E*	*C*	*l*	*L*
CD4-CD8+.....	1	2.33 ± 0.58	2.25 ± 0.50	0.058	
	2	2.00 ± 0.00	2.00 ± 0.00	0.000	6.476
	3	4.80 ± 0.45	3.60 ± 0.89	6.418	
CD4+CD8+.....	1	84.67 ± 1.53	83.00 ± 1.63	2.234	
	2	83.40 ± 1.14	81.20 ± 1.64	5.633	*8.145
	3	77.60 ± 1.52	74.40 ± 14.98	0.278	
Function:					
SI (ratio of cell numbers)	1	1.88 ± 0.12	1.72 ± 0.32	0.871	
	2	1.93 ± 0.31	2.09 ± 0.24	0.977	2.426
	3	1.78 ± 0.29	1.90 ± 0.24	0.578	
CTLa (%).....	1	59.00 ± 3.61	46.00 ± 13.93	2.729	
	2	45.60 ± 10.50	37.40 ± 7.37	2.273	7.318
	3	20.80 ± 4.32	25.20 ± 5.26	2.316	
NKa (%).....	1	16.67 ± 5.13	22.25 ± 2.22	4.086	
	2	9.20 ± 4.32	18.80 ± 4.87	8.581	*13.759
	3	29.00 ± 2.92	31.20 ± 4.21	1.092	

Note: SI, Stimulation index; CTLa, Cytotoxic T lymphocyte assay (E:T, 13:1); and NKa, Natural killer cell cytotoxic assay (E:T, 25:1).

*$P<0.05$

TABLE 5. Effect of Exposure to 60-Hz Magnetic Fields and Sham Exposure on Immune Parameters in Mice. Mice Were Exposed in a Series of Three pairs (Except 4 in the male 0.1-mT Experiment), Each Consisting of Five Exposed and Five Control Mice. The Effect of the Field was Evaluated Using the L Test. For Simplicity Only, the L Values of Pair-Wise Comparisons for Which *P* <.05 are listed (L > 7.83, except L > 10.20 for males at 0.1 mT).

	0.1 mT		0.5 mT		SHAM	
	Male	Female	Male	Female	A	B
Cellularity:						
Spleen	- - -	- - -	10.53	8.28	- - -	- - -
Thymus.....	- - -	8.00	- - -	- - -	- - -	- - -
Bone Marrow.....	- - -	9.59	- - -	- - -	- - -	- - -
Distribution:						
Spleen	- - -	- - -	- - -	- - -	- - -	- - -
CD45.....	- - -	11.58	- - -	- - -	- - -	- - -
IgM+	- - -	11.96	- - -	- - -	- - -	- - -
IgM+IgD-.....	- - -	30.54	- - -	- - -	- - -	- - -
IgM+IgD+.....	11.20	16.03	- - -	- - -	- - -	- - -
CD90+CD3+.....	13.08	11.40	- - -	- - -	- - -	- - -
NK1.1.....	- - -	- - -	12.10	- - -	- - -	- - -
Marrow:						
CD45.....	- - -	- - -	- - -	11.04	- - -	- - -
IgM+	- - -	- - -	- - -	- - -	- - -	- - -
IgM+IgD-.....	- - -	- - -	- - -	- - -	- - -	- - -
IgM+IgD+.....	9.783	- - -	- - -	- - -	- - -	- - -
Thymus:						
CD90+CD3+.....	13.15	8.17	- - -	12.26	- - -	- - -
CD4+CD8-.....	28.36	- - -	- - -	- - -	- - -	- - -
CD4-CD8+.....	10.18	- - -	- - -	- - -	- - -	8.86
CD4+CD8+.....	11.76	- - -	- - -	8.14	- - -	- - -
Function:						
SI	10.93	- - -	- - -	- - -	- - -	- - -
CTLa.....	13.39	- - -	- - -	- - -	12.51	- - -
NKa.....	11.73	19.54	25.67	13.76	- - -	- - -

Note: CD45 and CTL in Sham A were evaluated using only 2 pairs; *L* was adjusted to the equivalent 3-pair value. SI, Stimulation index. CTLa, Cytotoxic T lymphocyte assay (E:T, 13:1). NKa, Natural killer cell cytotoxic assay (E:T, 25:1). Sham groups A and B (arbitrarily designated) were both female.

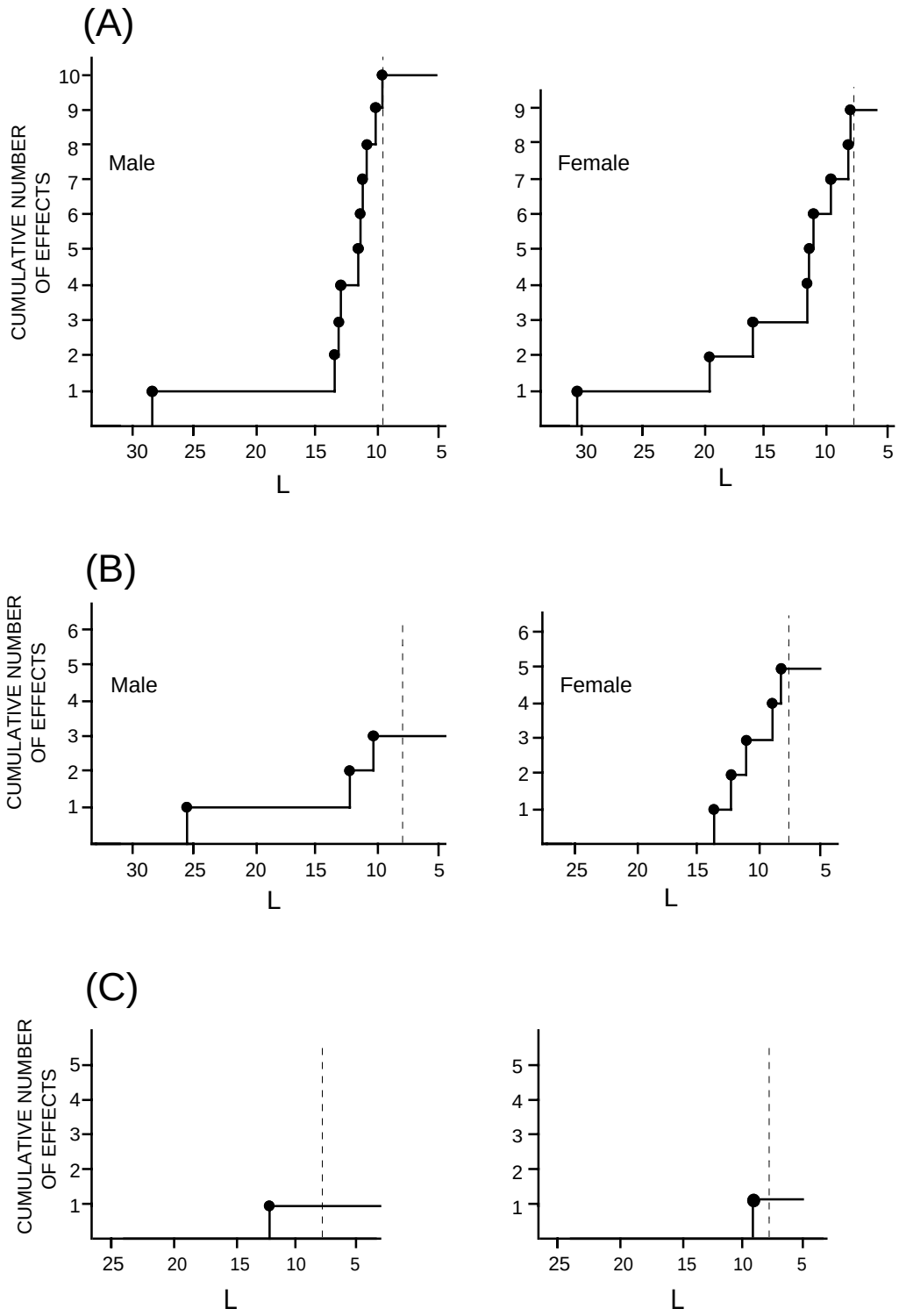

Fig. 1. Cumulative number of immune parameters (out of 20) that were significantly affected by EMF exposure as a function of the magnitude of the test statistic. **(A)** 0.1 mT. **(B)** 0.5 mT. **(C)**, two sham experiments (both females). Regions to the left of the dotted line indicate P <.05 (L > 7.83, except L >9.50 for males exposed to 0.1 mT).

TABLE 6. Comparison of Chaotic Systems Defined by the Lorenz Equations [Lorenz 1963]. The Parameters were chosen so that the System Operated in the Chaotic Mode ($\sigma = 16$, $b = 4$, $r = 45.92$), and the Equations Were Solved Using a Fourth-Order Runge-Kutta Method With a Time Step of 0.0125 s. Two Sets of Three Replicates Were Formed (N=5 From Each Set in Each Replicate) From the Transient Solution of One of the Three state Variables (Temperature; see Fig. 5). The Two Sets of Samples Were Obtained From Systems Having Initial Conditions $x_0 = 20$, $y_0 = 10$, $z_0 = 24$ and $x_0 = 20$, $y_0 = 10$, $z_0 = 26$, Respectively. L was Statistically Significant ($L = 9.01$, $P < 0.05$). No Difference was Found When L was Computed From the 15 Values Without Regard to Replicate Structure ($L = 2.45$, $P > 0.05$).

Rep. No.	24°C	26°C	l	L
1	5.78±10.69	5.34±10.96	0.07	9.01*
2	7.34±2.20	6.48±3.83	3.08	
3	1.76±8.45	4.91±8.03	5.86	

*$P < 0.05$

anomalous under an assumption that the underlying dynamical law is linear, but is lawful and may even be typical in the nonlinear case. How a nonlinear system might respond to an increased input by producing a decreased output is shown in Figure 5, which depicts the moving average for temperature in a Lorenz system. A heat input that elevated the initial temperature from 24 to 26°C resulted in a lower average temperature at various times during the evolution of the system. Determinism of this type is characteristic of nonlinear systems, and its occurrence even when the inputs are small is a hallmark of chaotic nonlinear systems. In these cases, the traditional concept of a dose-effect relationship is inapplicable.

Third, the percentile comparison plots (Figs. 2-4) showed that it was the animals at the tails of the distributions that were most affected by the fields. For example, the exceptionally high values for the exposed mice were greater than the exceptionally high values for the controls, and the reverse was generally true for the exceptionally low values. The concentration of the effect of the field at the tails of the distributions indicates that the interactions that gave rise to the differences were nonlinear in nature.

We previously reported that lymphoid phenotype of male mice was altered following exposure to 0.1 mT, 60 Hz for 21-105 days, and that the underlying dynamical law was

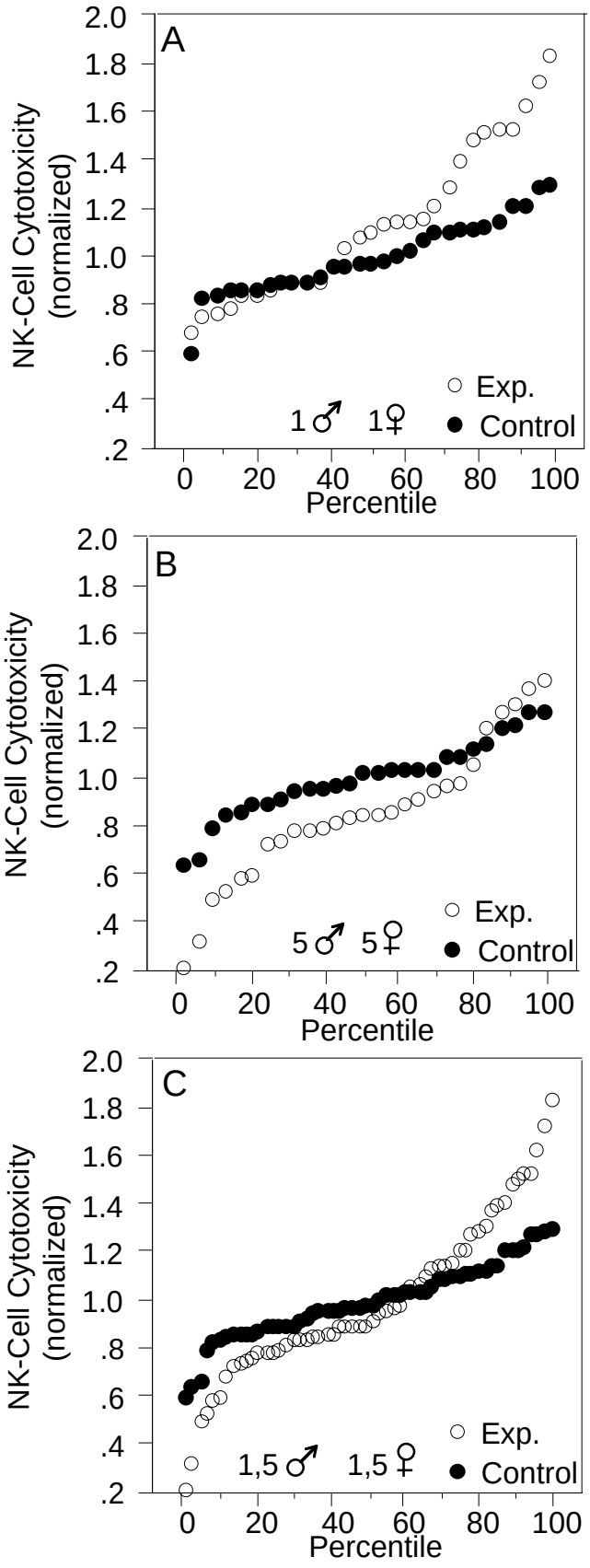

Figure 2. Effect of magnetic fields on NK-cell cytotoxicity. (**A**) 0.1 mT, males and females combined. (**B**) 0.5 mT, males and females combined. (**C**) A and B combined. Each point corresponds to one mouse. Statistically significant experiments (Table 5) are indicated by a gender symbol and field strength.

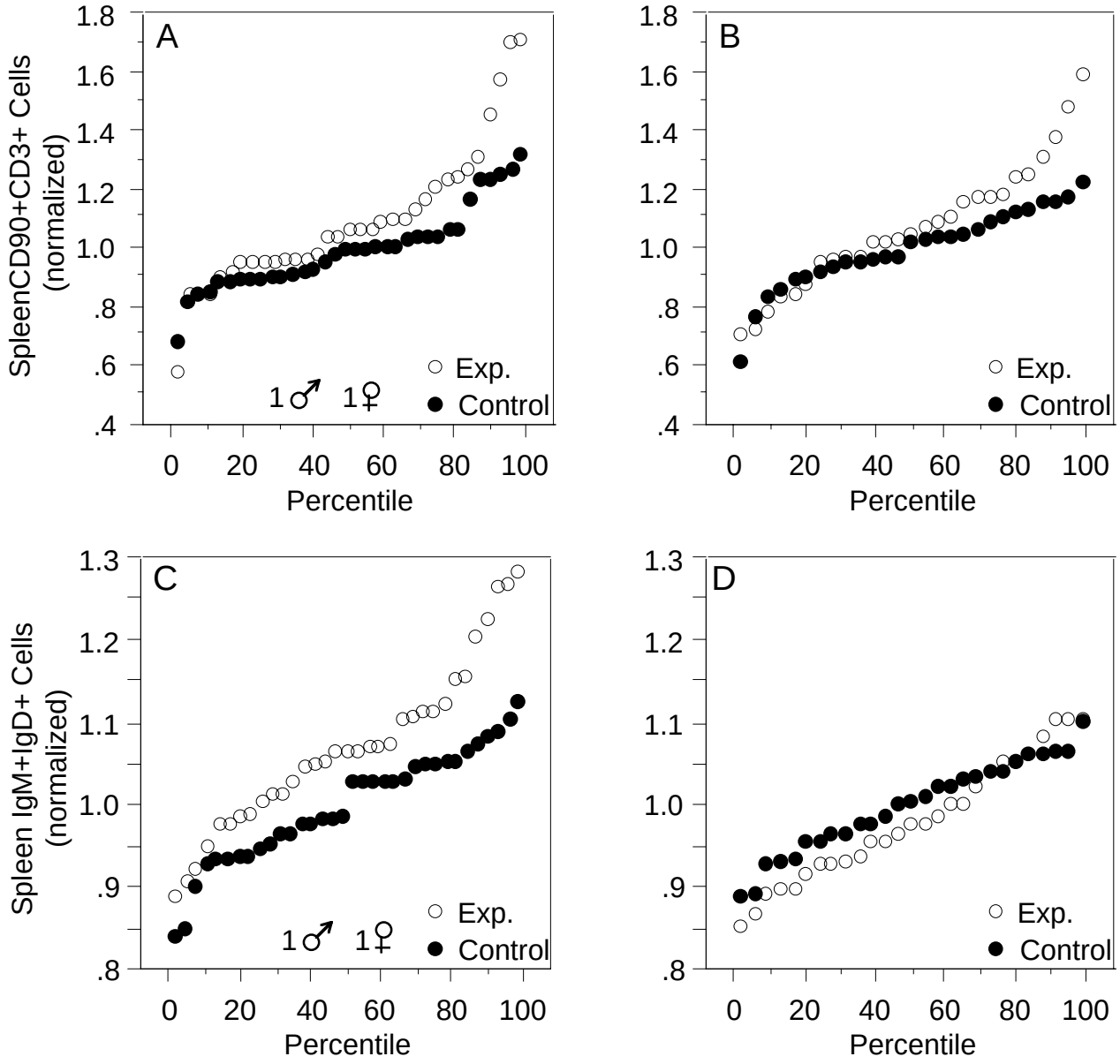

Fig. 3. Effect of magnetic fields on spleen CD90+CD3+ cells (**A** and **B**) and IgM+IgD+ cells (**C** and **D**). **A** and **C**, 0.1 mT, males and females combined. **B** and **D**, 0.5 mT, males and females combined. Each point corresponds to one mouse. Statistically significant experiments (Table 5) are indicated by a gender symbol and field strength.

nonlinear and likely chaotic [Marino et al., 2000]. On the basis of the considerations listed above, the conclusions of the earlier study may be extended to include both genders exposed at either 0.1 mT or 0.5 mT for 175 days.

Our finding that field exposure altered NK cell activity (Table 5, Fig. 2) deserves particular attention. We previously proposed a neuroendocrine theory (NET) to explain the link between EMFs and cancer [Marino and Becker, 1982; Marino, 1993]. According to the theory, EMFs are transduced in the nervous system, resulting in an afferent signal to the hypothalamus which then orchestrates adaptive electrical and hormonal responses. The magnitude and direction of the measurable parameters that constitute the response cascade are influenced in a nonlinear fashion by factors in the host and its environment. Chronic stimulation of the adaptive response taxes host immune defense thereby increasing the likelihood of cancer. NK cells (large granular non-B non-T lymphocytes) are of particular interest in this regard because of their role in

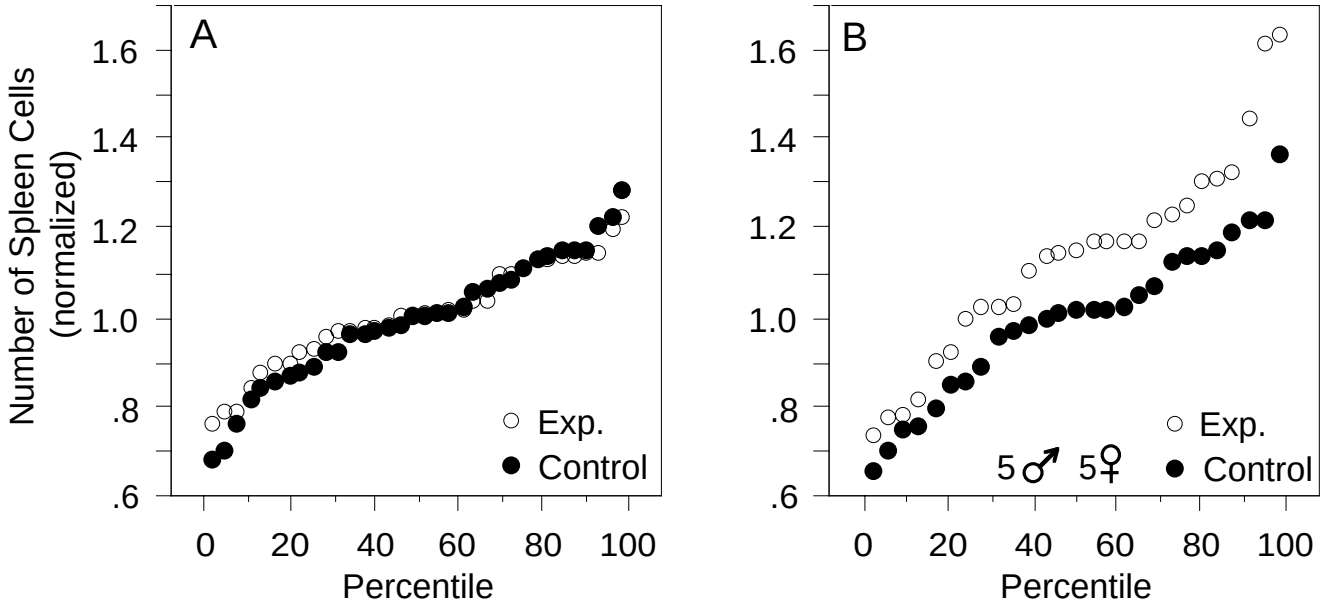

Fig. 4. Effect of magnetic fields on number of spleen cells. (**A**) 0.1 mT, males and females combined. (**B**) 0.5 mT, males and females combined. Each point corresponds to one mouse. Statistically significant experiments (Table 5) are indicated by a gender symbol and field strength.

mediating natural immunity [Ortaldo and Herberman, 1984]. It is possible that the epidemiological association between EMFs and cancer predicted on the basis of the NET arises from EMF impaired immunosurveillance. If so, an effect of field exposure on NK cell numbers or function would be expected. We showed previously that NK cell cytotoxicity in male mice was significantly affected following exposure to 0.1 mT, 60 Hz for 49 and for 105 days of exposure

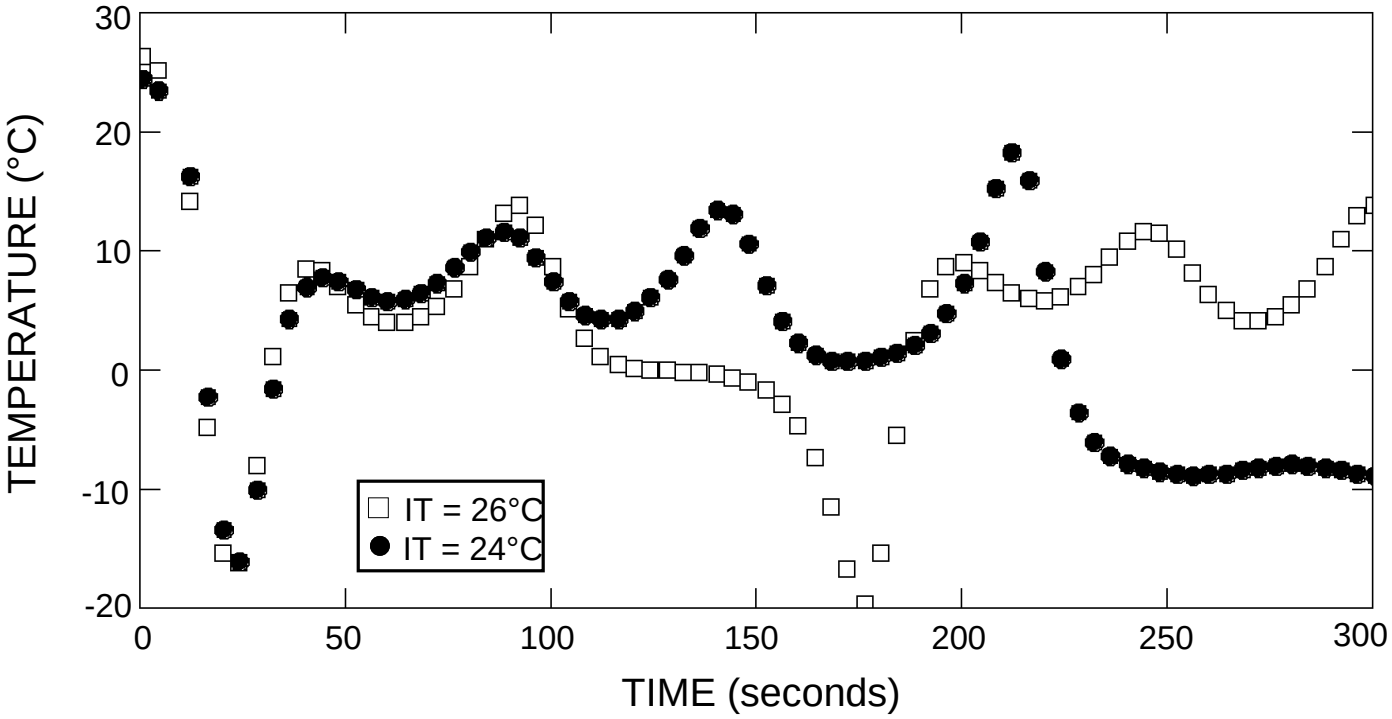

Fig. 5. Moving average for temperature in the Lorenz system. IT, initial temperature (N=5 for each point).

[Marino et al., 2000]. In the present study, a similar effect on NK cells was found in all four experiments (Table 5). Overall, therefore, our research supports the hypothesis that the link between EMF exposure and cancer is mediated by impaired immunosurveillance.

It remains to be seen how, and to what extent, controversies regarding mechanisms, replicability, and the existence of specific biological effects [Olden, 1999] can actually be resolved by allowing nonlinearity. Similarly, the EMF exposure conditions under which NET might be a useful mechanistic framework remain to be established. Finally, the basic biophysical process that characteristically mediates transduction of power-frequency fields and the anatomical location of the interaction remain to be ascertained. What seems clear, however, is that power frequency field biological determinism is fundamentally nonlinear, at least as manifested in the immune system.

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