Different outcomes in biological experiments involving weak EMFs: is chaos a possible explanation?

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Marino, Andrew A. Different outcomes in biological experiments involving weak EMFs: is chaos a possible explanation? *Am. J. Physiol.* 268 *(Regulatory Integrative Comp. Physiol.* 37): R1013—R1018, 1995.—Reports of biological effects induced by electromagnetic fields (EMFs) are sometimes not confirmed in subsequent similar studies. It is possible that the fields in these studies were actually transduced by the biological systems and that the pattern of apparently conflicting results is not a valid inference but arises from the conceptual framework. The question was studied in connection with effects of EMFs on body weight of animals. Previous studies revealed no consistent effect on mean body weight, but reexamination of the data suggested that variance in body weight was consistently altered after long-term exposure. In the present study, 0.5 *kVIm,* 60 Hz, was applied continuously from conception to maturity in successive generations of mice; as hypothesized, mean body weight was not consistently affected but variance in body weight was altered. The occurrence of transduction can therefore be inferred from the effect on variance despite the absence of a consistent effect on the mean. These results and those from previous similar studies support an interaction model in which the observability of posttransduction biological changes in the mean is strongly dependent on the initial conditions, in analogy with the importance of initial conditions in fixing the final state in deterministic chaos. Thus, at least with regard to EMF effects on body weight, a change from a linear (dose-effect) to a nonlinear paradigm is sufficient to explain the anomalous pattern of some reported studies.

variance; transduction; biological effects

ANIMAL STUDIES IN THE 1970's suggested that exposure to weak electromagnetic fields (EMFs) caused various biological effects, and interest in the area increased after therapeutic applications and findings of associations between environmental EMFs and cancer (3). Sometimes, however, reports of field-induced biological effects were followed by related studies in which the previous effects were not observed (1, 2, 5, 8, 13, 17, 21, 23, 27, 29—31), thereby casting doubt on whether the effects were real and raising the issue of why EMF effects have not always been reproducible in all laboratories.

One possibility is that biological systems exhibit a nonlinear response to EMFs that simply appears random when the means of biological responses are measured in typical laboratory studies. If, after transduction, the magnitude and direction of an EMF-induced biological effect were strongly influenced by small differences in the initial conditions, then the nonreproducibility of some results might be explained on the basis of a failure to exactly recreate the initial conditions. Such a sensitivity to the initial conditions is the distinctive feature of deterministic chaos (9). Both chaos and chance are consistent with the absence of reproducible changes in the mean, but only chaos suggests a reproducible effect on variability in the parameter being measured.

Many studies of the effect of EMFs on body weight of chronically exposed animals have been performed (10, 11, 14-16, 19, 22, 24-26, 28), with the overall result of no consistent effect. Despite this, an analysis of the studies suggested that EMF exposure consistently resulted in increased variability in weight irrespective of the absence of changes in mean weight. This study was therefore undertaken to prospectively evaluate the relationship between EMF exposure and variance in body weight. The aim was to verify that the fact of EMF transduction (the seminal effect attributable to an EMF) could validly be inferred despite the absence of a consistent effect on the mean. The exposure parameters, environmental conditions, and animal handling procedures were chosen on the basis of previous studies (14).

METHODS

Ha/ICR mice were exposed to vertical or horizontal electric fields, with separate controls for each group to ensure the appropriate comparability of housing conditions. One housing unit held both the horizontal-exposure (HE) and horizontal-control (HC) groups, but separate units were built for the vertical-exposure VE and vertical-control (VC) groups 3, 14). The horizontal unit accommodated 45 cages, each situated between a pair of vertical metal plates; by appropriately grounding or energizing the plates, exposure (0.5 kV/m) and control environments could be located next to one another. This design afforded some protection against the possible role of unrecognized environmental factors, but it resulted in some fringing field at the control locations. Each vertical unit consisted of three parallel-plate capacitors that could each accommodate up to nine cages. In the VE unit, 0.5 kV/m was produced in the intra-plate regions; in the VC unit, all plates were grounded and the ambient 60-Hz electric fields were eliminated by wrapping the cage-containing regions with grounded aluminum screening. The screening reduced the light levels inside the control unit by 10%, and consequently the VE unit was wrapped with fiberglass screening that had light-transmission properties identical to those of the aluminum screening.

The three units were housed in a windowless room that contained filtered nonrecirculated air that had not been in previous contact with other animals. The room was exposed to a minimal amount of corridor traffic, cage washers, and other sources of noise. A 12:12-h light-dark cycle commenced at 0600; the average temperature was 22CC, and the humidity was 40-50%. The respective 60-Hz electric and magnetic fields at the location of the cages from sources other than those controlled in the study were 2—15 *VIm* (NC groups only, estimated from calculations of the fringing field of nearby conductors) and 0.1—0.4 mG (from current flow in equipment wiring, measured with a calibrated pick-up coil). The positions of the three units were interchanged weekly to randomize the

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uncontrolled microenvironmental factors. The immediate environment of the animals was totally nonmetallic: the cage bedding material was commercially purchased sanitized wood chips. and feeding and watering were ad libitum.

Initially, two females were bred to one male, with a minimum of 15 breeding groups in each of the four treatment conditions. When pregnancy occurred (the F_1 generation), the male and the second female from each group were removed and the pregnant females were caged individually. Each litter was reduced to six randomly selected animals within 1 day after birth to lessen the influence of litter size on growth; litters with fewer than six animals were not used. The females remained with their offspring until weaning, at which time the offspring were separated by sex and the female was removed. When the mice were ~3 mo old, randomly selected individuals from the F_1 generation of each group were mated (brothersister mating was precluded) and similarly produced and reared their offspring. Randomly selected individuals from the second (F_2) generation then produced the third (F_3) generation. Thus, the HE and VE groups were continuously (>23.5 h/day) exposed to a 60 Hz, 0.5 kV/m field imposed in the horizontal and vertical directions, respectively, from conception to maturity, and the HG and VC groups were raised in their respective ambient 60-Hz fields. The number of animals that survived to maturity and the resulting body weights were measured.

The F-test was used to compare variances in the exposed and sham-exposed mice (6). The assumption that the data were normally distributed was tested with the Kolmogorov Smirnov test (20). A total of 12 F-tests were performed, corresponding to 2 fields x 3 generations x 2 sexes. The test statistic was $F = s_1^2 / s_2^2$ where s_1^2 was the larger sample variance in each comparison. The two-tailed rejection region of the F distribution for a single comparison was $F > F_{0.025}$, which corresponds to a probability for a type-1 error of 0.05 (18). The family-wise error rate was controlled by using the Bonferroni procedure (12) to adjust the comparison-wise error to be 0.05/12 0.0042, which corresponded to a rejection region of $F > F_{0.002}$. Because this approach involves no assumption of independence, it was applied to weight data obtained at periodic intervals throughout the growth period as well as to body weight at maturity.

RESULTS

More than 93% of the mice in each group that were alive on the first day after birth survived the exposure period, and the survival rates were not affected by the electric field (Table 1). The overall results of the effect of electric-field exposure on body weight are summarized in Fig. 1, which shows the mean body weight of each exposed group expressed as a percent change from its corresponding control group. In some instances, apparently consistent changes in weight with time were observed, e. g., the F_3 generation males exposed to the vertical or horizontal electric field, but there were comparable numbers of instances in which field exposure tended to increase body weight (males and females exposed to the vertical field, F_2 generation) or resulted in no consistent changes (males and females exposed to the horizontal field in the F_1 generation). Overall, there was no discernible pattern regarding the effect of the electric field on body weight of the exposed animals. However, when the variance of the data was examined, evidence indicating an effect due to the electric field was found. Table 2 shows the body weights and SDs at the end of the study *(days 96-105,* depending on the generation) of each exposed group and its corresponding control group. After controlling for the family-wise error, the occurrence of one or more significant tests for which $F > F_{0002}$ would have been sufficient justification to accept the hypothesis that the field exposure affected variance, and two such cases were observed. Furthermore, when data comparable to that in Table 2 but obtained during the study were analyzed, one or more significant cases $(F > F_{0.002})$occurred at each of the four prior times at which body weights were ascertained (Table 3).

Table 1. *Survival (to last day) in 3 generations of mice exposed to 0.5 kV/m 60-Hz electric fields*

Generation	Treatment	No of Litters	No. of Surviving Mice		%Survival
			Day 1	Last Day	
F_1	VE	17	102	95	93
	HE	15	95	89	94
	VC	19	104	101	97
	HC	16	101	99	98
F_2	VE	16	96	92	96
	HE	12	72	70	97
	VC	14	84	78	93
	HG	14	87	86	99
F_3	VE	20	120	112	93
	HE	20	107	101	94
	VC	20	120	115	96
	HG	20	108	105	97

VE, vertical exposure; HE, horizontal exposure; VC, vertical control; HG, horizontal control. Last day was *day 105* for F_1, *day* 96 for F_2, and *day 98* for F_3.

DISCUSSION

Exposure of mice to 60-Hz electric fields was previously found to alter mean body weight in successive generations (14), but inconsistent effects were found by others. In one study, EMF-exposed males and females were smaller than the sham-exposed control animals, but the opposite results (exposed animals heavier compared with control animals) were observed in a replicate of that experiment (22). In another study, growth retardation was observed in the F_2 generation but not the F_1 generation in each of two experiments (24). Overall, therefore, the studies provided no consistent evidence that the exposure caused biological changes. However, if the data from these studies are analyzed with the variance in weight as the dependent variable—and not mean body weight as chosen by the investigators—the implications are quite different. Three generations of mice exposed to 3.5 kV/m, 60 Hz in the vertical or horizontal direction (14), three generations of mice exposed to 100 kV/m, 60 Hz applied vertically in both the initial experiment and its replicate (22), and two generations of mice exposed to 0.82—23 gauss, 50 Hz (24) each exhibited significantly altered variance compared with the respective control animals. These results suggested that variance in body weight was consistently affected by long-term exposure to low-frequency EMFs, and the results of the present study confirmed that

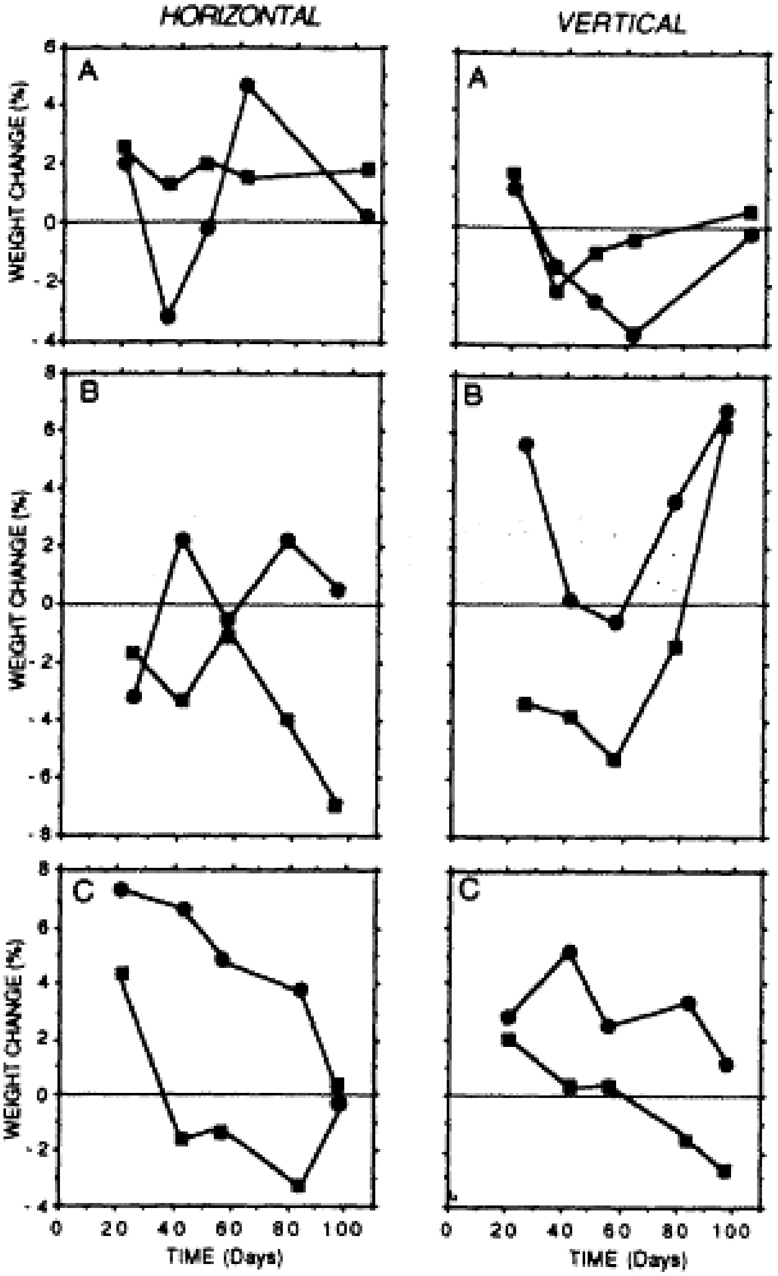

Fig. 1. Influence of 60-Hz horizontal and vertical electric fields (0.5 kV/m) on mean weight. Results are expressed as %change relative to mean of corresponding control. A: F_1 generation. *B:* F_2 generation. *C:* F_3 generation. =, males; <, females.

Table 2. *Body weight of mice exposed to 60-Hz horizontal or vertical electric fields at 0.5 kVIml*

		Horizontal Field			Vertical Field		
Generation	Sex	HC	HE	*F* Value	VC	VE	*F* Value
F_1 (l05days)	M	40.8±4.8 (47)	40.9±4.4 (47)	1.19	43.2±4.5 (54)	43.1±5.3 (53)	1.39
	F	31.4±3.8 (52)	32.0±4.3 (42)	1.28	31.6±2.6 (47)	31.8±4.9 (42)	3.55
F_2 (96 days)	M	37.4±6.8 (42)	37.6±4.3 (34)	2.50	36.4±3.4 (37)	38.9±3.8 (47)	1.25
	F	29.3±3.4 (44)	27.1±3.9 (36)	1.32	26.7±3.1 (41)	28.4±3.2 (45)	1.06
F_3 (98 days)	M	38.8±4.2 (53)	38.7±4.2 (45)	1.00	37.2±4.6 (66)	37.6±2.5 (60)	3.38*
	F	28.9±4.1 (52)	28.9±4.0 (56)	1.05	28.5±3.3 (49)	27.7±2.3 (52)	2.06

Values are means ± SD where indicated; no. of mice is given in parentheses. $*F > F_{0.002}$ ($P < 0.05$).

Table 3. *Cases (out of 12 for each measurement) for which variance in body weight in exposed and sham-exposed groups differed significantly ($F > F_{0.002}$)*

	Measurement No.				
	1	*2*	*3*	*4*	*5*
Days	21—25	35—42	49—56	63—84	96—105
Protocol	Males, F_2, HE	Females, F_1, VE Females, F_3, VE	Females, F_1, VE Females, F_2, HE	Females, F_3, VE	Females, F_1, VE Males, F_3, VE

First measurement was made at *days 21, 25,* and *21* of exposure in F_1, F_2. and F_3 generations, respectively, and variance in 1 group (HE F_2 generation males) differed significantly. Comparable data for 4 additional measurements (each made within the indicated time interval) are also shown. Occurrence of ≥1 significant difference at a particular measurement was sufficient to reject null hypothesis at that measurement time.

inference. Irrespective of the absence of a consistent effect of EMF on mean body weight (Fig. 1), the fields caused a change in variance when assessed at the end of the exposure (Table 2) and when assessed at various times during exposure (Table 3). Thus, the conclusion that might otherwise be drawn from the observation of no consistent effect on mean body weight, namely, the absence of signal transduction, should be rejected in favor of the conclusion that signal transduction must have occurred, since variance was significantly affected. The possibility that some factor other than the field was responsible for the consistent effect on variance found in the various multigenerational studies cannot be completely discounted, but it seems unlikely because the studies were performed by different investigators under different circumstances. Any such putative factor would have had to be present to roughly a similar degree in all studies.

In addition to EMF exposure over successive generations, other exposure durations have been employed in previous studies, including conception to birth, conception to weaning, and specific intervals ranging from 1 mo to 1 yr (10, 15, 16, 19, 25, 26, 28). Largely negative effects on mean body weight were reported in these studies, but, again, that was not the case with variance (Table 4). Four of the six studies manifested statistically significant differences, indicating an overall trend toward an impact of exposure on variance. It is improbable, based on the binomial theorem, that so many successes would be observed by chance *(P* < 0.05). The published reports are therefore generally in accord that chronic exposure to EMFs of different field intensities altered the variance in body weight in different species at various times during development.

The results here and in previous studies (10, 11, 14-16, 19, 22, 24-26, 28) suggest an interaction model in which the transduction of EMF actually occurs, leading to a biological consequence with regard to body weight that is critically dependent on the initial conditions, including internal state of each animal, analogous to the chaos model in which the response of the system is bounded but not precisely predictable because of its marked dependence on the initial conditions. Similar concepts have been expressed by many other investigators, including Frey (who described the implausibility of the toxic-effects model) (7) and Cannon (who distinguished the roles of "seed" and "soil" in the elaboration of a biological effect) (4). The common feature of these models is the assumption of nonlinearity of response, in

Table 4. *EMF effects on variance in body weight of mammals, as determined from previous studies*

Species	Ref. No.	EMF	Exposure Duration	Experiment No.	Sex	Body Wt., g		F Value	P Value
						Control	EMF		
Pigs	28	30 kVm, 60 Hz	Conception to birth	*1*	**M**	536 ± 74.2 (28)	553 ± 157.5 (56)	4.50†	<0.001
					F	510 ± 91.7 (29)	518 ± 135.0 (56)	2.16†	0.015
				2	M	576 ± 129.2 (29)	532 ± 109.3 (71)	1.40†	0.130
					F	573±123.8(29)	488±118.0'(71)	1.10†	0.36
Monkeys	10	2G,20V/m,72—8OHz	lyr		M	2,290±510(14)	3,060±470*(14)	1.18	0.39
					F	1,290±700(16)	1,260±920)16)	1.73	0.15
Rats	25	150 kV/m, 60Hz	Conception to 21 days		M	47 ± 6.7 (56)	45 ± 13.7 (58)	4.18†	<0.001
					F	43 ± 8.2 (56)	44 ± 12.9 (58)	2.47†	<0.001
Rats	26	8OkV/m,6OHz	Conception to weaning	*1*	M	66.5±31.1 (123)	65.6±35.4(148)	1.29	0.070
					F	60.8±29.4 (119)	59.4±25.8 (126)	1.30	0.075
				2	M	45.1±27.9(268)	42.9±40.0(220)	2.06	<0.001
					F	42.7±20.6(295)	42.7±31.2(270)	2.29	<0.001
				3	M	41.7± 16.4 (188)	41.9±29.6(199)	3.25	<0.001
					F	38.9±15.7(204)	41.3±28.8(208)	3.36	<0.001
Rats	19	0.1kV/m,45Hz	36days		M	414±17(47)	362±9~(47)	3.57	<0.001
Rats	15,16	0.1kV/m,45Hz	28days	*1*	M	398.5±30.1(16)	395.9±40.6(16)	1.82	0.13
				2	M	349.1 ± 29.3 (16)	358.1 ± 25.5 (16)	1.32	0.30
				3	M	398.6±34.2(16)	388.3±21.3(16)	2.58	0.038

Studies that used low-frequency fields and presented sufficient data to permit *F* test are included. Values are means ± SD where indicated; no. of animals is given in parentheses. EMF, electromagnetic field. Rejection region for *F* is $P < 0.025$, which corresponds to probability of type 1 error of <0.05. **S** significantly different compared with control, $P < 0.05$. † Contains round-off error due to uncertainty in sample size.

distinction to the assumption of linearity that is sometimes invoked (11).

How is it possible to view an inconsistent observation on body weight in causal terms? Let W_i be the weight of the ith animal, and assume that it is absolutely determined by the instantaneous value of the variables, x_j, where j = 1, 2,...J. Some x_j values are exogenous, such as temperature, and some are endogenous, such as the level of enzyme X; the x_j values depend on time and on each other. The *i*th and *k*th animals are selected because they are identical with respect to all internal and external factors that affect body weight; the *k*th animal is exposed to an EMF for time T, whereas the *i*th animal is maintained in an identical but field-free region. If EMF exposure caused an increase in enzyme X which, in turn, caused an increase in body weight, we could validly identify EMF as the cause of the increase in body weight. However, the ultimate effect on body weight due to the change in enzyme X induced by EMF will also depend on the particular combination of values of the J-1 variables other than enzyme X. An identical effect on enzyme X might occur in each of a group of reasonably homogeneous animals exposed to EMF, but an identical effect on body weight will not necessarily occur because, in general, the animals will differ from one another with regard to the instantaneous value of each non-X variable. Thus, EMF may increase or decrease body weight or cause no change at all; such changes may sum to zero in a particular group of animals, but each change biases toward an effect on sample variance. In this manner, by allowing an animal response to be determined by both its outer environment (which can be controlled by the investigator) and its internal environment (which is not well controlled), EMF causality can be reconciled with apparent inconsistency.

In summary, the present evidence suggests that deterministic chaos provides a useful conceptual framework for modeling biological interactions associated with EMF effects on body weight and perhaps other EMF-induced biological effects. Chaos does not require imposition of a condition (change in mean) for accepting an event (transduction) as real.

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