NONLINEARITY IN BIOLOGICAL SYSTEMS: HOW CAN PHYSICS HELP?

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Physical theory can explain all events known to occur in the inanimate world. In stark contrast, theory cannot explain or predict any behavior in even the simplest living organisms. The reason living systems are essentially unpredictable is that their interactions with factors in the environment are governed by nonlinear dynamical laws. When appropriate analytical methods are employed, it is possible to routinely observe the nonlinear determinism manifested by biological systems.

The present fashion in biology, which is to pursue the study of mechanisms but ignore the dynamical laws that drive them, is inadequate for approaching many significant scientific problems. To solve them it will be necessary to think like a physicist, that is to think in terms of mathematical laws that govern the system under consideration. But it is not a physicist rooted in the traditions of the past that is needed, where essentially all the objects of study and analysis were linear systems governed by equations directly traceable to the deep laws of physics. Physics in the context of biology must be reconceptualized so that probabilistic explanations and short-term predictions based on heuristic laws are seen as the ultimate obtainable goal.

1. Introduction

Physical theory has undergone incredible development within the past 2 centuries, resulting in what arguably is mankind's greatest intellectual achievement. Our present knowledge of the laws

governing gravity, electromagnetism, and atomic forces, and bow these laws function in different size and velocity domains is almost universally thought to be complete. We expect that any observation in the inanimate world can be explained by the equations of motion of the system from which the observation was drawn.

No similar success has been achieved in understanding the animate world. The great advances in biology in the last half century have mostly involved discovery, rather than explanation. Thus, we now know about complex processes in the brain and immune systems, cytokine signaling networks, and the genetic basis of health and disease. Despite these discoveries, we have no understanding of biological activity in the sense that we can explain it deductively from the basic laws of nature. We cannot predict the future of any aspect of any biological system to a reliability that remotely parallels the reliability of the predictions that can be made about the behavior of nonliving things. Indeed, given all the great laws of physics, it is not even possible to predict that there is such a thing as life.

One explanation for our present lack of understanding of how individual biological systems function in time is that our ignorance reflects a lack of attention to the problem, and that with a more concentrated effort we will achieve the ability to explain and predict the activities manifested by living systems. A problem with this viewpoint is that biology is clearly not developing in the direction of mathematization of observations and theories, which is a requirement for prediction as well as the best evidence of understanding. On the contrary, the great institutions where biology is taught now produce graduates who have essentially no training whatever in mathematics, and no inclination to think about biological systems in mathematical terms. The emphasis in these institutions is on observation and manipulation for purposes of elucidating mechanisms that mediate biological processes, rather than on understanding the dynamical laws that operate these mechanisms.

Another possibility that could explain why we lack the ability to predict how living organisms will behave is that they are governed by as yet undiscovered laws. What makes this explanation particularly unappealing to me is that it assumes living organisms arc fundamentally different — not just more complex — than the rest of nature. and I cannot find any evidence to support that proposition.

The explanation I propose is that, in many of their important manifestations, living organisms are similar to a kind of deterministic physical system that is unpredictable because it is nonlinear. In other words, because of the way living organisms are constructed, they exhibit behaviors that cannot be anticipated, at least when measured against the benchmark for predict-

ability furnished by linear systems. My purpose here is to present the evidence for this thesis, and to discuss its implications for the role of physics in biology.

In the next section I distinguish linear and nonlinear systems and illustrate their key differences. It is an empirical question whether biological systems are like one or the other, and in the following section I discuss the conditions necessary to make such a judgment based on observation. I then show that, when the appropriate conditions are satisfied. biological systems can be recognized as fundamentally non linear in nature.

Data does not speak for itself, but rather takes meaning from its context, especially including the model employed in its analysis. I present two cases illustrating how the arbitrary assumption that the biological system under study was linear in its ability to respond to a stimulus markedly altered the meaning of the data. The examples illustrate the necessity of avoiding the error of hypostatizing biological systems as linear systems.

Finally, I offer a view regarding how the project of physics, which I take to be an effort to provide the best possible explanation of nature in natural terms, can be carried out most profitably in the domain of living systems. I think physics in the context of biology must be reconceptualized so that probabilistic explanations and short-term predictions based on heuristic laws are seen as the ultimate attainable goal.

2. Physical Systems

By a “linear system” I mean a system governed by linear differential equations. Linear systems obey the law of superposition, thereby ensuring that outputs will be proportional to inputs, and will be completely predictable. Essentially all man-made machines are linear systems for the obvious reason that there is no utility in an airplane, bicycle, cell telephone, automobile, or any other system that did not function the same way each time its initial conditions were duplicated. In this sense, linear systems display no novelty. This is a key point. and I want to illustrate its implications at the level of observation.

A spring is a prototypical example of a linear system (Figure 1) The equation of motion of a mass, M, at the end of a spring having a constant k is:

$$M\ddot{y} + k_y = 0.$$

The general solution is

$$y = c_1 \cos at + c_2 \sin at$$

where $k/M = a^2$. The arbitrary constants C_1 and C_2 can be determined from the initial conditions $y(0)$, $Y(0)$. The solution reveals the fact that the spring vibrates forever with a simple harmonic motion whose period is $T = 2\pi/a$.

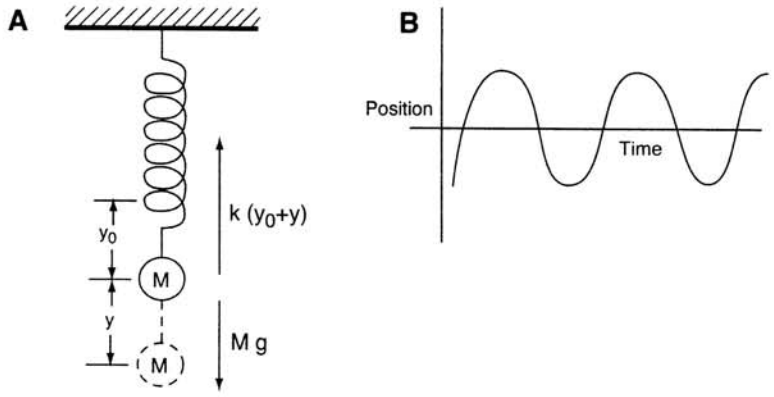

Figure 1. Motion of a mass oscillating in a vacuum at the end of a spring. The mass (M) stretches the spring at equilibrium by a distance y0. If the mass is further displaced and released, its motion is determined by Hooke's law and gravity.

Even if we had no knowledge of gravity, Hooke's law, or Newton's law, we could still observe the behavior of a spring exhibiting the oscillatory motion depicted in Figure 1B and, on the basis of observation alone, predict the behavior of the system indefinitely far into the future.

A "nonlinear" system is a system governed by nonlinear differential equations. Although man does not often build such systems, nature does — a premier example is the weather. A second key point is that observations of some nonlinear systems may not allow prediction of their future behavior. To see how this can be the case, consider the system of three ordinary differential equations derived by Lorenz to describe thermal conduction in the atmosphere [1].

$$\dot{x} = \sigma(y - x).$$

$$\dot{y} = -xz + rx - y,$$

$$\dot{z} = xy - bz$$

where x, y, and z are the system variables (two temperatures and a velocity) and σ, r, and b are system parameters. A particular solution for x in the so-called chaotic mode is shown in Figure 2. In contrast to the obvious pattern displayed by the linear system, the pattern of the chaotic system is completely inobvious in time; observations of a chaotic physical system, therefore, would not permit prediction of the system's future better than a guess.

The nonlinear system is remarkably different from the linear system in another way — sensitivity to initial conditions. After about 10 seconds the perturbed and unperturbed systems (Figure 2) exhibit dramatically different behaviors even though the distinction that constituted the perturbation (10^{-6}°C) is too small to resolve experimentally.

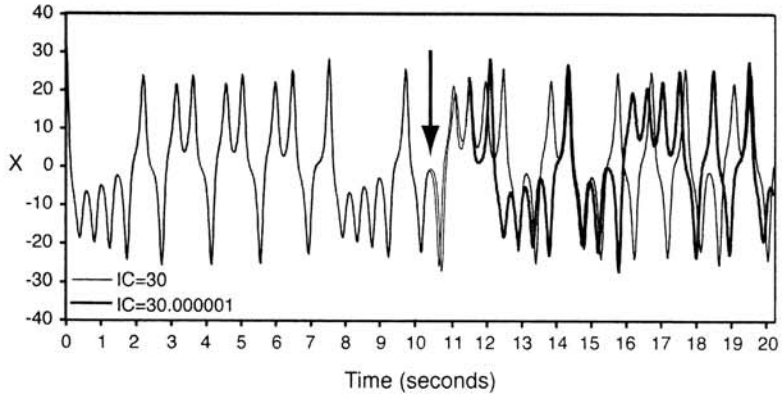

Figure 2. Time series of one variable from the Lorenz system of equations (I). The thin line corresponds to an initial condition x(0) = 30.000000. The heavy line corresponds to the initial condition x(0) = 30.000001. The solutions separate after about 10 seconds (arrow). Solution method, fourth order Runge-Kutta algorithm, integration step = 0.01; σ = l6, r = 45.92, b = 4.

3. Biological Systems

By a “biological system” or a “living system” I mean a normally metabolizing unicellular or multicellular system that produces an output when subjected to an input. Probably the chief difference between physical and biological systems is the great complexity of the latter. The simplest object that could arguably be considered to be living is a virus whose genome codes for only 3 proteins [20]. Despite its simplicity when considered as a living system, the virus is infinitely more complex than any object studied by physicists in the laboratory.

By a “biological effect” I mean a relationship that can reasonably be modeled in terms of an input/output relationship (Figure 3). My thesis is that observed input/output relationships in biological systems are frequently nonlinear (sensory transduction may be a major class of exceptions). I will not discuss the character of the dynamical laws that govern the internal operation of a living system. I believe they also are nonlinear, but a discussion of the empirical evidence supporting this view would take me too far from my goal, which is to describe what I think is the proper role for physics in biology.

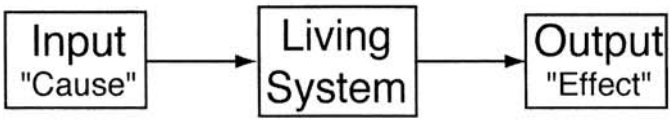

Figure 3. A “biological effect” is defined as an output of a metabolically driven system subjected to an input.

The great laws of physics are linear, and the myriad man-made systems that surround us have been designed to operate under linear principles. Appropriately, the methods used to analyze the output of these systems are linear. Thus, discrete outputs are typically analyzed by comparing their means, and time-series outputs are analyzed using analytical techniques such as the fast Fourier transform. It is easy to think of these methods as broadly applicable to any system, linear or nonlinear. I now want to show that this is not the case, and therefore that special methods are required to permit reliable observation of biological activity.

Suppose we have 5 more-or-less identical living systems, say 5 laboratory rats, and the instantaneous values of a particular immune parameter are considered to be their outputs. The outputs are measured while the animals are in a normal (“control”) condition, following which they are exposed to a stimulus and then re-measured. We can distinguish 3 possible results (Figure 4).

If the input-output relationships are linear, then the output observed from each animal will be consistent, changing by a roughly similar amount in each animal.

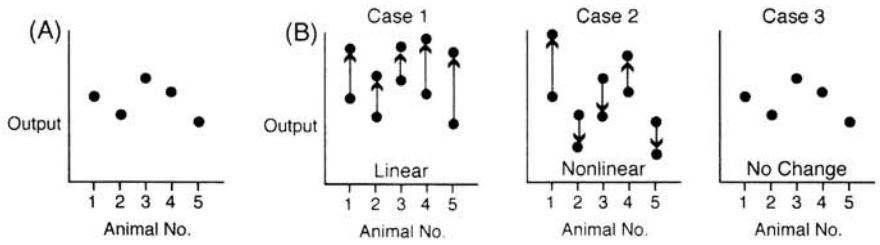

Figure 4. Possible responses in a group of 5 rats subjected to the same input. (A), output levels measured immediately prior to the input. (8), outputs measured immediately after the input.

However, if the input-output relationship is governed by nonlinear dynamical law, as we have seen (Figure 2), the instantaneous output at any particular time is inconsistent in the sense that different animals not precisely in phase (which is obviously the situation for living systems) would be recorded as exhibiting increases or decreases, compared with the levels observed prior to application of the input. The experimental problem is to distinguish cases 2 and 3 which, on average, appear identical.

One way in which a nonlinear effect might be recognized would be to evaluate the change in the variance of the data following application of the input. It is easy to see, for example, that the variance in case 2, Figure 4, exceeds that of the other cases. Consequently, a statistical test based on comparison of variance could evidence a nonlinear response, even in the absence of a change in the mean (means in cases 2 and 3 are identical by hypothesis). Note that if the means in cases 2 and 3 were each compared with the control, using the t test, for example, the deterministic effect depicted in case 2 would not be recognized. On the other hand, if the observations were as shown in case I, the underlying determinism could be recognized on the basis of the t test. These considerations show that analyzing a nonlinear system using a method suitable for analyzing a linear system can lead to a false conclusion that no effect occurred.

Studies where the input was an electromagnetic field (EMF) well illustrate these points. For example, when I evaluated the effects of EMFs on body weight of animals, as reported in studies published between 1975–1995 [7], I found consistent, statistically significant effects on variance even though there was no consistent effect on the mean (Table 1) [5,12–14,17–19].

Table 1. EMF effects on variance in body weight of mammals. The studies that used low-frequency fields and presented sufficient data to permit an F test are included. The means ± SD are listed; the number of animals is given in parentheses. M, male; F, female. The F value and the corresponding probability are listed in the last two columns. The rejection region for F is $P < 0.025$, which corresponds to a probability of type-1 error of < 0.05 [7].

Ref. No.	Species	EMF	Exposure Duration	Exp. no.	Sex	Body Weight (gms)		F	P
						Control	EMF		
19	Pigs	30 kV/m 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
5	Monkeys	2 Gauss	1 year		M	2290 ± 510 (14)	*3060 ± 470 (14)	1.18	0.39
		20 V/m 72–80 Hz			F	1290 ± 700 (16)	1260 ± 920 (16)	1.73	0.15
17	Rats	150 kV/m 60 Hz	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
18	Rats	80 kV/m 60 Hz	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
14	Rats	0.1 kV/m 45 Hz	36 days		M	414 ± 17 (47)	*362 ± 9 (47)	3.57	<0.001
12, 13	Rats	0.1 kV/m 45 Hz	28 days	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

Perusal of the biological literature will reveal many instances in which the variance of the output of the biological system under study was affected by the input, but where that biological effect was not recognized by the investigators because a linear model was assumed.

Studies involving the immune system provide another example. All reported studies of the effects of EMFs on the immune system had assumed the applicability of a linear model, resulting in inconsistent results that led to the overall conclusion that the immune system was not affected by fields [16]. We conducted a series of prospective studies to test the hypothesis that the response of the immune system of mice to EMFs was governed by nonlinear laws.

It was necessary to develop a method of statistical analysis to test our hypothesis. For a nonlinear system, an effect caused by an input would not be observed by comparing means in large samples because oppositely-directed change would be averaged away (Figure 4B, case 2). Small samples might reveal nonlinear effects due to incomplete averaging, but statistical tests on small samples lack statistical power. To overcome this problem. we developed a novel statistical procedure for inferring the occurrence of nonlinear effects, based on the likelihood approach. This approach allows differences in means from replicate series of exposed and control groups to be combined to test an overall hypothesis, in our case that EMFs affected the immune system. The log-likelihood ratio of the *t* statistic for a *t* test between an exposed and control group is

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

where N is the number of animals in each group. The distribution of l is approximately chi-square with I1 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 chi-square 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.

A result typical of those found in our immune studies is shown in Figure 5, which depicts the number of statistically significant effects on the immune system that were found following exposure to 60-Hz, 1 G, as evaluated on the basis of $L > \chi^2_{4,0.05}$, N = 5. Half of the immune parameters measured were significantly altered as a consequence of EMF exposure, which was far greater than the number that could be explained on the basis of chance. The alternative hypothesis of consistent change in the immune data (which would suggest the applicability of a linear model) was evaluated by combining the individual measurements in the 4 replicates prior to analysis ($L > \chi^2_{1,0.05}$, with N=20) (equivalent to performing a t test on the combined data). In this case we observed only the number of significant changes expected on the basis of chance. Thus, it was possible to detect a nonlinear relationship between the input and the output of the biological system when it was modeled nonlinearly, but not when it was modeled linearly. Further details regarding Land the results obtained using it to examine the effects of EMFs on the immune system are presented elsewhere [8–11].

When it is possible to obtain time-series output data from a living system, the problem of discriminating between cases 2 and 3 (Figure 4) is simplified because of recent developments in nonlinear signal analysis.

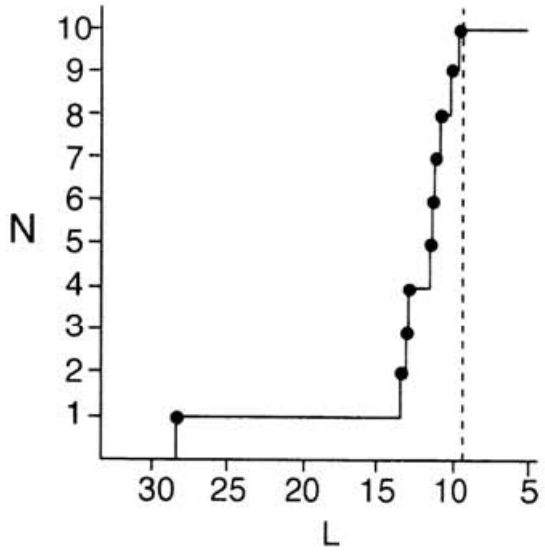

Figure 5. Cumulative number of immune parameters (N) (out of 20) in male mice that were significantly affected by exposure to I G, 60Hz, as a function of the magnitude of the test statistic, assessed on the basis of whether $L > \chi^2_{4,0.05}$ and N=5. Region to the left of the dotted line indicates P < 0.05. Control experiments showed that, at most, one of the statistically significant changes could be attributed to chance. The immune parameters measured were: cellularity in spleen, thymus, and bone marrow; percent distribution of lymphocyte subpopulations in the spleen (CD45, IgM+, IgM+IgD–, IgM+IgD+, CD90+/CD3+, NK1.l), marrow (CD45, IgM+, IgM+IgD–, IgM+IgD+), and thymus (CD90+/CD3+, CD4+CD8–, , CD4-CD8+, CD4+CD8+); stimulation index; cytotoxic T lymphocyte assay; natural killer cell cytotoxic assay [10].

Using phase-space methods [6], time-series data that appears irregular in the time domain (Figure 2) and that appears to be colored noise in the frequency domain (Figure 6A) can exhibit a recognizable pattern when viewed in phase space Figure 6B). Such patterns can be visualized using recurrence plots (4] that can be quantitated using recurrence quantification analysis (RQA) [21–23].

We used nonlinear methods to analyze the electroencephalogram from rabbits exposed to EMFs (Figure 7). Typical results are shown in Figure 8, as assessed using two RQA quantifiers [12]. Using nonlinear methods we were able to prove the existence of a nonlinear relationship between an applied EMF an d a change in the brain's electrical activity which occurred in every animal studied.

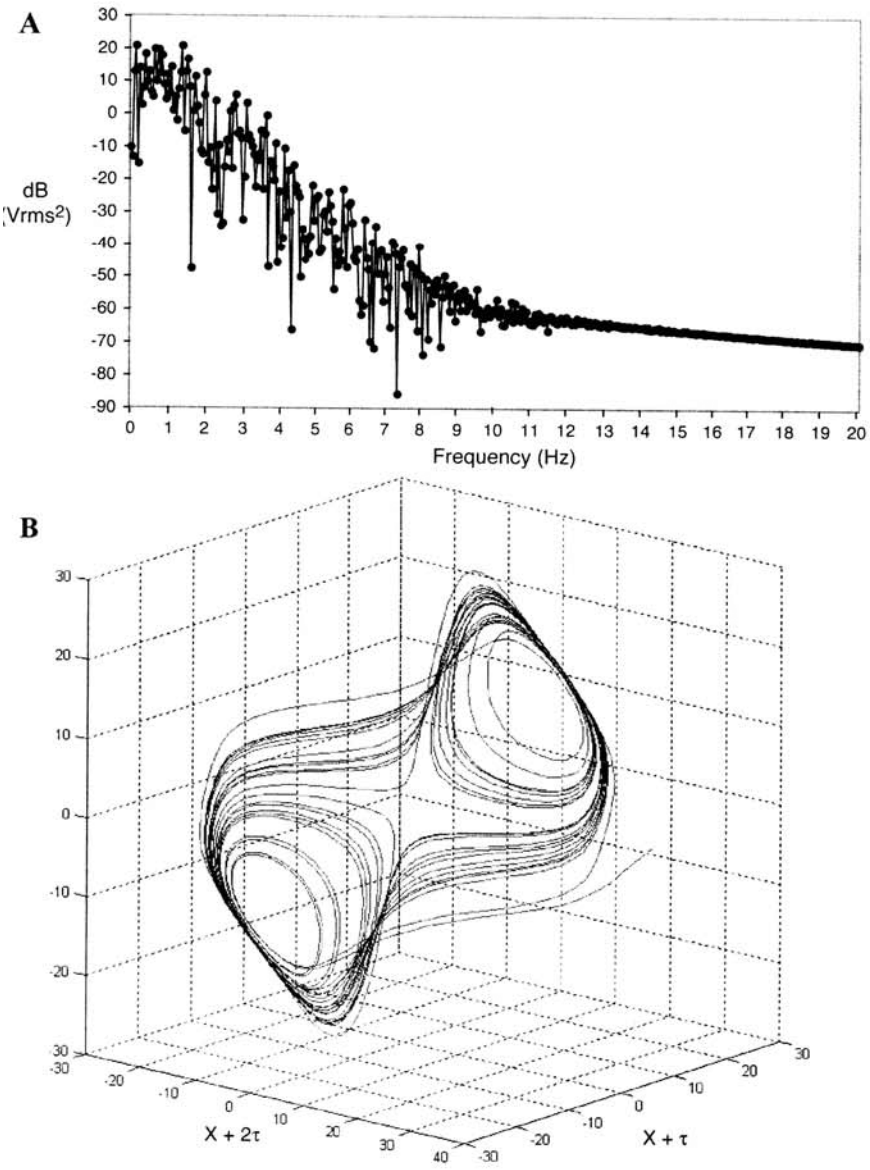

Figure 6. Appearance of the solution of the Lorenz equation (Figure 2, IC = 30) in the frequency domain (A) and in phase space (B). τ (time delay) = 0.1 sec; embedding dimension = 3.

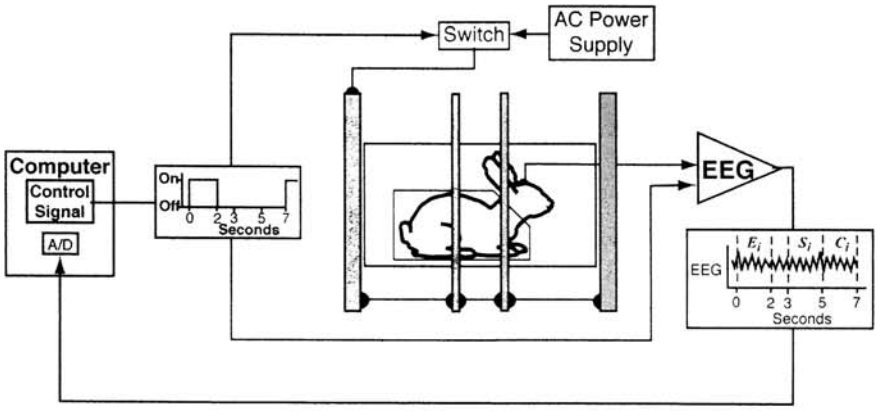

Figure 7. Schematic representation of the experimental system used to expose rabbits to 2.5 G, 60 Hz and assess the effect on the electroencephalogram. A computer-generated timing signal controlled switching of the stimulus. The timing signal was also fed into one of the channels of the EEG amplifier to facilitate identification of the exposed (E), sham (S), and control (C) epochs of the EEG in each trial (the ith trial is illustrated). The results of 50 trials were analyzed using the Wilcoxon signed-rank test. The location of the rabbit relative to the field-producing coils (shaded bars) is shown.

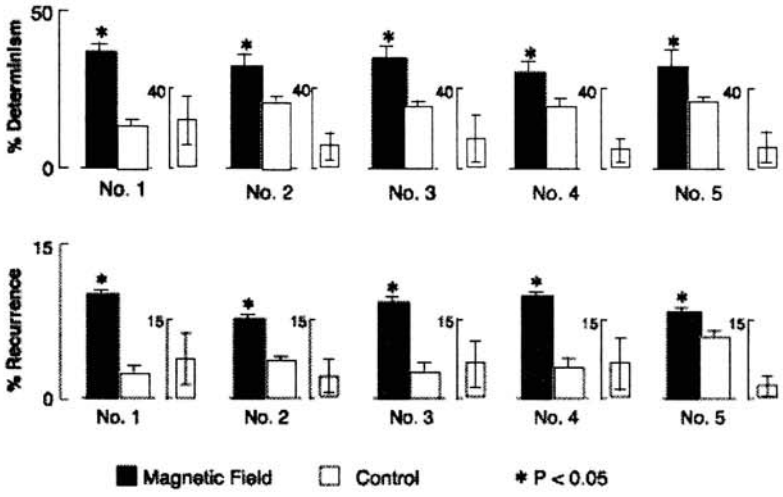

Figure 8. Effect of 2.5 G, 60 Hz in 5 female rabbits, as assessed using two RQA quantifiers. For each rabbit and each quantifier, the difference between the exposed and control EEG epochs was evaluated using the Wilcoxon signed-rank test. The average values of the quantifiers (±SD) and the 95% confidence limits of the test metric are presented for each rabbit [12].

Taken together these examples show that when appropriate analytical methods are employed it is possible to routinely observe nonlinear determinism in biological systems.

4. Sensitivity to Initial Conditions

I want to present two examples of how failing to recognize nonlinearity in the input-output relationships of biological systems can lead to an incorrect picture of how the living system responded to the input.

Figure 9 depicts the results of 2 independent, controlled experiments involving exposure of mice to EMFs. Each experiment consisted of mating and rearing the mice while they were exposed to the field [15]. The offspring of the first generation (F_1) were mated to produce a second generation (F_2), and they were used to produce the final (F_3) generation; for each generation, the mean body weights at maturity were compared with their controls. In one experiment the investigators found that the exposed mice in the first generation were statistically significantly lighter than the corresponding controls (about 8% on average). The difference was not statistically significant in the second generation, but in the third generation the difference was again statistically significant.

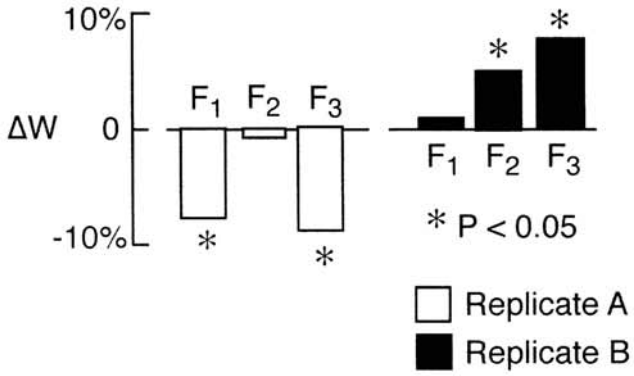

Figure 9. Replicate experiments of the effect of EMF (100 kV/m, 60Hz) exposure on growth in 3 generations of mice (F_1–F_3) [15]. ΔW, EMF-induced change in mean body weight relative to control. The data presented is for male mice. Similar results were found for the females.

In their second experiment, the investigators found that the second and third generations were statistically significantly heavier than the corresponding controls (Figure 9).

One way to interpret these results (the proper way, I suggest) is to regard the basic relationship between the applied field and growth as being nonlinear in nature, and exhibiting sensitivity to initial conditions. In this view, unascertained (and hence uncontrolled) environmental conditions that differed between the two experiments caused the oppositely directed effects. This view leads to the conclusion that field exposure affects body weight but that the direction of the effect cannot be predicted.

An alternative explanation (which was the position adopted by the investigators) is that only linear effects can occur and, since linear effects must be consistent, observation of apparent inconsistent effects in the two experiments indicated that there was no biological effect.

Another example of how the failure to recognize nonlinearity leads to incorrect conclusions is provided by the so-called Henhouse studies, a group of identical experiments that were carried out in six different laboratories. In 1982, Delgado and colleague [3] had reported that EMFs caused skeletal abnormalities in chicken embryos. The report led to many follow-up studies, some of which confirmed the effect and some which did not. One proposed hypothesis to explain the apparent inconsistencies was to assume that they were due to differences in the exposure systems used in the studies, and that if everyone used exactly the same apparatus and procedure. consistent results would be obtained. The exposure systems were therefore rigorously standardized and similar experiments were carried out in three laboratories in the United States and three in Europe. Overall, the result was dismissed as negative [16], even though significantly more defective embryos were found among the EMF- exposed eggs in 2 of the 6 laboratories (Table 2) [2].

These two examples clearly revealed the role of sensitivity to initial conditions in altering the manifestation of the effect of the EMF. If neither group of investigators could eliminate the impact of sensitivity to initial conditions, despite great efforts and the expenditure of millions of dollars, it is safe to conclude that it cannot be done. As I showed in the previous section, sensitivity to initial conditions is a fundamental, defining property of nonlinear systems.

Table 2. Proportions of normal living embryos (mean ± SE). Approximately 100 embryos in the EMF and in the control group were studied at each laboratory. On the basis of ANOVA, there was a significant difference between the EMF and control groups, $F(1,54)= 12.09$, $P = 0.001$. *$P < 0.05$ [2].

Laboratory Location	*Sham-Exposed*	*Exposed*
London, Ontario, Canada	*0.936 ± 0.024	0.794 ± 0.024
Umeă, Sweden	0.916 ± 0.026	0.874 ± 0.026
Rockville, MD, USA	*0.903 ± 0.030	0.778 ± 0.030
Madrid, Spain	0.829 ± 0.041	0.796 ± 0.057
Chapel Hill, NC, USA	0.784 ± 0.027	0.785 ± 0.035
Las Vegas, NV, USA	0.730 ± 0.050	0.699 ± 0.044

5. Role of Physics

It is undoubtedly true that the deep laws of physics apply with full force and effect to both living and nonliving systems. But living systems are vastly more complex than any of the systems studied thus far by physicists in the laboratory. For this reason, the deep laws of physics are of almost no help in producing useful information concerning the future of specific biological systems. The situation is analogous to a game of chess in which one player knows the basic rules regarding the movement for each piece, but nothing about the strategy of the game. Even perfect knowledge of the basic rules will not save that player from disaster in the absence of heuristic knowledge regarding what kinds of moves make sense in particular situations. These heuristic rules are emergent properties of chess and do not exist at the same organizational level as the basic rules. It is this idea that I think is critical to an understanding of the role of physics in modem biology. The deep laws are not alien to biology, they are simply not sufficient to explain the observed behavior of any particular biological system. The best that can be done is to study the biological systems empirically with the aim of developing heuristic explanations. It is necessary to recognize that Laplacian determinism is a dream, unachievable in practice, and to settle for probabilistic knowledge that is better than a guess but far from the precision with which linear systems can be characterized.

The present fashion in biology is to study mechanisms and eschew consideration of dynamical laws. I think this strategy is inadequate for approaching a whole range of biological problems that most people consider important and that are intrinsically dynamic, not structural.

How do factors that cause cancer or other disease work? What guides embryonic development? How is pain transmitted or blocked? When will strokes or heart attacks occur in particular individuals? What is life?

To solve these and other important problems I think it will be necessary to think like a physicist, that is to think in terms of mathematical laws that govern the system under consideration. But it is not a physicist rooted in the traditions of the past that is needed, where essentially all the objects of study and analysis were linear systems governed by equations directly traceable to the laws of physics. Physics in the context of biology must be reconceptualized so that probabilistic explanations and short-term predictions are seen as the ultimate attainable goal.

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