Physical Mechanisms Underlying Neurite Outgrowth: A Quantitative Analysis of Neuronal Shape

F. Caserta,[1] H. E. Stanley,[1] W. D. Eldred,[2] G. Daccord,[3] R. E. Hausman,[2] and J. Nittmann[4]
[1]*Center for Polymer Studies and Department of Physics, Boston University, Boston, Massachusetts 02215*
[2]*Department of Biology, Boston University, Boston, Massachusetts 02215*
[3]*Dowell Schlumberger, 42003 St. Etienne, France*
[4]*Digital Equipment Corp., Vienna 1040, Austria*
(Received 22 June 1989)

We show that—over a range of length scales r—the shapes of quasi-two-dimensional retinal neurons are fractal objects, and hence may be quantitatively characterized in part by their fractal dimension d_f. We analyze the shapes of numerous retinal neurons, both *in vivo* and *in vitro*. The neurons *in vivo* are found to have a fractal dimension d_f of 1.68 ± 0.15. We also propose an explanation of certain stages of neuronal shape development in terms of a diffusion-limited-aggregation model, which predicts $d_f = 1.70 \pm 0.1$.

PACS numbers: 87.10.+e

Neurons in the central nervous system, and the retina in particular, have a characteristic morphology—a cell body from which radiate processes (neurites) termed the neuronal arborization.[1] The ability to identify a neuron based on the qualitative shape of its arborization has long been recognized. However, meaningful quantitative analyses remain elusive. The shape attained by a neuron is thought to result from *environmental* as well as genetic influences.[2] Many local environmental effects, such as growth factors and electrical charge, are known to influence these directional choices.[3,4]

The critical question is how these "local" effects result in the complex branching pattern of a neuron, in contrast to the behavior of, say, a phototropic plant like *Phycomyces* which adopts a branchless structure when grown in the presence of a point source of light.[5] Complex branching patterns in other kinds of growth[6,7] are based on diffusion-limited processes, and are quantitatively described using fractal analysis.[6-9] The diffusion-limited-aggregation (DLA) model has recently been shown to model both outgrowth and aggregation processes.[8,9]

A fractal shape can be completely described by a single parameter, d_f, the fractal dimension (the mass of a fractal object inside a radius r scales as r^{d_f}). Objects in nature are fractal over a finite range of length scales r, typically a factor of 10 or so. Here we apply fractal analysis to retinal neurons *in vivo* and *in vitro*. We find that neurons are fractal objects over roughly a decade in r. We also suggest three possible diffusion-limited processes that could be related to the fractal shapes observed.

Photographs of the neurons were digitized with a video camera, using a grid of $2^{16} = 65\,536$ pixels. The fractal dimension d_f of the digitized patterns was determined as follows using the box-counting method. First we compute the center of gravity and radius of gyration. Then we take as the origin one point within a square centered at the center of gravity and with a side equal to the radius of gyration. Every point on the structure, within this square is chosen as a local origin and the cluster mass (number of occupied pixels) within a distance r of this local origin is calculated. Averaging over all possible choices of local origin, including the empty sites, we find the averaged cluster mass $M(r)$ scales with r as

$$M(r) \sim r^{d_f}. \tag{1}$$

Thus the slope of a double logarithmic plot of $M(r)$ against r gives a quantitative value of d_f. We also used the correlation method to calculate d_f and obtained similar values of d_f. Both the box-counting and correlation protocols have been successfully applied to a wide range of fractal objects.[6,7] We have chosen the retina as a model system because it contains many neurons with unique dendritic arborizations that lie primarily in two dimensions (with an aspect ratio of approximately 10:1). This both facilitates analysis (minimizing complicating effects arising from growth in a third dimension) and allows comparisons to culture conditions. Our analysis of retinal neurons *in vivo* in adult animals shows neurons with well developed axons and dendrites. In contrast, our *in vitro* studies show neurons where axon and dendrite growth is not yet well developed.

(i) In vivo.—Figures 1(a) and 1(b) show retrogradely labeled β ganglion cells from the cat retina.[10] Figures 1(c) and 1(d) are the corresponding double logarithmic plots of $M(r)$ against r, using box counting, which—over a tenfold range of r values—are as linear as most fractal objects in nature. The falloff at large values of r is customary for *all* fractal shapes, and corresponds to the fact that for sufficiently large r some shells are larger than the entire pattern. From the slopes of the linear portions we estimate $d_f = 1.71$ and 1.69 for Figs. 1(c) and 1(d), respectively. Averaging over all the patterns of eleven neurons *in vivo*, we find $d_f = 1.68 \pm 0.15$ by box counting and $d_f = 1.66 \pm 0.08$ by the correlation method.

(ii) In vitro.—As a first step in trying to analyze the relative contributions of electrical, trophic, and viscosity

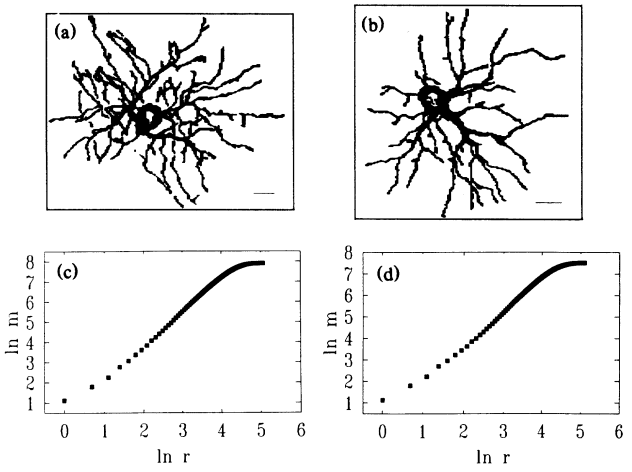

FIG. 1. (a),(b) Digitized images of retrogradely labeled β ganglion cells in a cat retina (Ref. 10), taken at early and later stages of development, respectively. The scale bar is 10 μm. (c),(d) The corresponding fractal analyses.

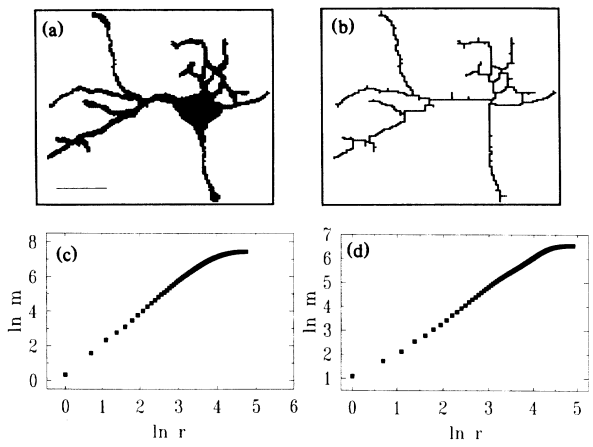

FIG. 2. Digitized image of a chick retinal neuron *in vitro*. Retinal cells were obtained, cultured for 14 d, and photographed according to Ref. 11. (a),(b) Digitizations of the neuron and of the "skeleton" (see description in text) of the same neuron, respectively. The scale bar is 10 μm. (c),(d) The corresponding fractal analyses.

factors in producing these fractal patterns, we studied neurons which developed in a radically modified environment: retinal neurons grown under known conditions *in vitro*. Cells were prepared and maintained in culture according to Hausman *et al.*[11] Figure 2(a) is typical of one of the eleven neurons analyzed using the methods described above. The average value obtained for d_f of the *in vitro* neurons is 1.43 ± 0.1 by box counting and 1.39 ± 0.1 by the correlation method [Fig. 2(c)]. These *in vitro* values are significantly different from those obtained *in vivo*.

The *in vitro* neurons are truly two dimensional, while the *in vivo* neurons have some three-dimensional character which complicates the interpretation in terms of models. Moreover, the analysis of all neuronal patterns is more complex due to nonuniform branch width (the patterns present a decreasing branch width as one goes away from the cell body). A similar effect occurs in chemical dissolution patterns,[7] where one finds a decreasing branch width as one moves away from the injection point.[12] We corrected for this effect by forming the "skeleton" of the pattern [Figs. 2(b) and 2(d)], removing all points from the digitized image that are not necessary for the global connectivity.[12] Analysis of the skeletized images yielded more appropriate values of d_f than those found for the original patterns, since skeletizing eliminates the cell body ($d_f = 2$). Using the correlation method which is relatively insensitive to the cell body, we found almost identical values of d_f for the original image and the skeleton, thus demonstrating that the parameter d_f is fairly insensitive to pattern "details" such as branch width.

Significantly, we find using both box counting and the correlation method that the 22 neurons analyzed are fractal objects. For the *in vivo* neurons, $d_f = 1.68 \pm 0.15$ by box counting. This value for the fractal dimension is the same as that found for the diffusion-limited-aggregation model, a model that has been found to describe a vast range of "diffusion-limited" phenomena.

The DLA model incorporates two features that might apply to neurite outgrowth at the molecular level: (i) The factors controlling the growth are those inherent in a diffusion equation, and (ii) the growth proceeds by *stochastic* growth rules. Concerning point (i), we note that at least three diffusion-limited physical processes which may result in neurons having fractal shapes are electrical fields, chemical gradients, and viscosity differences. Two of these, electrical fields and chemical gradients, are known to modify the shape of neurons.[3,4]

To explain point (ii), we show in Fig. 3 the first few steps in the DLA growth process. The key point is that the growth follows rules that faithfully represent the solution of the equations for a diffusion-limited process, *including the presence of stochastic noise.* It has recently become established that the resulting clusters accurately describe a class of growth phenomena in which the diffusion equation or Laplace equation $\nabla^2\phi = 0$ controls the essential physics. Thus growth phenomena governed by chemical gradients (in which case ϕ is the concentration), by electrical gradients (ϕ is an electrical potential), or by a difference in viscosity between the inside and outside of the pattern (ϕ is the pressure) are all believed to be described by the DLA model.[8,9] It is known that growing neurons respond to chemical gradients and electric fields, and it is also known that there is a difference in viscosity between the neuronal cytoplasm and the surrounding intercellular matrix. For this reason, it is not implausible that DLA might represent a zeroth-order description of neuron growth.

The value of d_f for *in vitro* neurons is not as easily attributable to the DLA model. Obviously, *in vitro* envi-

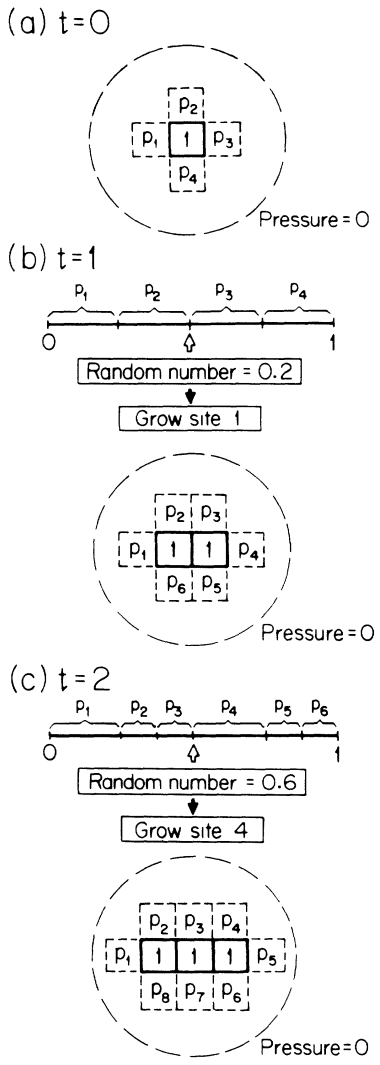

FIG. 3. Schematic illustration of the first steps in the generation of a DLA cluster by solving directly (on a square lattice) the Laplace equation $\nabla^2\phi=0$, where ϕ represents the concentration, electrical potential, or pressure (if growth phenomena are governed by chemical gradients, electrical gradients, or a difference in viscosity between the inside and outside of the cell, respectively) (Refs. 8, 9, and 21). At time $t=0$ we place a seed particle at the origin. We calculate the solution to the Laplace equation everywhere, with the boundary conditions that $\phi=1$ on the seed and $\phi=0$ on a large circle that surrounds the system. We assign probabilities to the perimeter sites of the seed particle chosen in proportion to the value of $\nabla\phi$, and partition the unit interval such that each probability corresponds to one segment of that interval. Next we choose a random number between 0 and 1. The value of the random number chosen determines which site will grow; in the example shown, this is the site labeled p_1. We now have a two-site cluster. This process is iterated; i.e., the Laplace equation is solved again, a set of six probabilities for the six perimeter sites is placed in 1:1 correspondence with the unit interval, a new random number is chosen (in this case 0.6), and the corresponding perimeter site is grown (in this example, site 4). By this rule, one accurately obtains diffusion-limited aggregates of large size.

ronmental influences would be considerably modified from those *in vivo*. If such environmental factors influence the fractal nature of neurons, then it is quite reasonable to expect changes in d_f as these factors are changed.

Considerable further study is needed to see if a particular d_f value can be associated with a particular cell type in terms of its anatomy, physiology, or developmental state. To properly address this intriguing possibility, fractal dimensions would have to be calculated for a large number of identified cells to determine if there is a statistically significant relationship between functional cell type and fractal dimension. An important step in this direction has been taken by Amthor, who analyzed the fractal dimensions of *directionally selective* retinal neurons.[13]

In summary, many biological phenomena appear to be fractal,[14,15] including for example the chick embryo circulatory system,[16] structure of the bronchial tree,[17-19] and the human retinal circulatory system;[20] the present study is among the first to systematically partly characterize a biological *structure*, the fully developed retinal neuron *in vivo*, using fractal mathematics (using both box counting and the equivalent correlation function method). Moreover, the reproducibility of the numerical values for d_f of fully developed neurons *in vivo* suggests that d_f is *not* a parameter devoid of physical content; indeed, for nonbiological objects, accurate measurements of d_f have led to subsequent physical understanding.[21]

After completing this work we learned that Smith *et al.* also measured d_f for unspecified vertebrate central-nervous-system neurons in culture.[22] Our study is primarily towards the comparison of measurements *in vivo* (in the adult animal) where the environment in which the neurons grow is complex and measurements *in vitro* where the environment through which the neurons grow is considerably simpler and more defined. In these *in vivo* studies, which we believe to be the first to be reported, we find a d_f of 1.68 ± 0.15 and we build an analogy with DLA. Therefore, our work is complementary to that of Smith *et al.*; however, we go on to suggest that a known growth process (DLA) may in part underlie this value of d_f. Our present work presents quantitative descriptions of neuronal shape, and suggests an underlying physical basis. This should allow subsequent mathematical analyses of the underlying biophysical processes.

The work at Boston University was supported in part by grants from the NSF, ONR, and NEI. We thank Professor H. J. Herrmann and Professor L. Liebovitch for constructive criticism, and Professor B. Hoop for helpful discussions. This work is based in part on the Ph.D. thesis of F.C.

[1]S. Ramón y Cajal, *The Structure of the Retina* (Charles Thomas, Springfield, IL, translated 1972).

[2]R. O. Lockerbie, Neuroscience **20**, 719–729 (1987).

[3]R. W. Gundersen and J. N. Barrett, Science **206**, 1079–1080 (1979).

[4]L. F. Jaffe and M.-M. Poo, J. Exp. Zool. **209**, 155–128 (1979).

[5]See, e.g., E. P. Fischer and C. L. Lipson, *Thinking About Science: Max Delbrück and the Origins of Molecular Biology* (W. W. Norton & Co., New York, 1988).

[6]J. Nittmann, G. Daccord, and H. E. Stanley, Nature (London) **314**, 141–144 (1985); G. Daccord, J. Nittmann, and H. E. Stanley, Phys. Rev. Lett. **56**, 336–339 (1986).

[7]G. Daccord, Phys. Rev. Lett. **58**, 479–482 (1987); G. Daccord and R. Lenormand, Nature (London) **325**, 41–43 (1987).

[8]T. Vicsek, *Fractal Growth Phenomena* (World Scientific, Singapore, 1989).

[9]J. Feder, *Fractals* (Plenum, New York, 1988).

[10]J. Maslim, M. Webster, and J. Stone, J. Comp. Neurol. **254**, 382-402 (1986).

[11]R. E. Hausman, M. S. Katz, E. T. Dobi, and J. Offermann, Int. J. Develop. Neurosci. **4**, 537–544 (1986).

[12]G. Daccord, Phys. Rev. A **39**, 1365–1368 (1989).

[13]F. Amthor, Society for Neuroscience **1988**, 603 (abstract).

[14]L. Liebovitch, in "Advanced Methods of Physiological Systems Modeling," edited by V. A. Marmarelis (Plenum, New York, to be published), Vol. II.

[15]P. Meakin, J. Theor. Biol. **118**, 101 (1986).

[16]A. A. Tsonis and P. A. Tsonis, Perspect. Biol. Med. **30**, 355 (1987).

[17]B. J. West, Bull. Am. Phys. Soc. **34**, 716 (1989).

[18]B. J. West and A. L. Goldberger, J. Appl. Physiol. **60**, 189 (1986).

[19]B. J. West and A. L. Goldberger, Am. Sci. **75**, 354 (1987).

[20]F. Family, B. R. Masters, and D. E. Platt, Physica (Amsterdam) **38D**, 98 (1989).

[21]Applications are described in many recent review articles. See, e.g., P. Meakin, in *Phase Transitions and Critical Phenomena,* (edited by C. Domb and J. L. Lebowitz (Academic, Orlando, 1988), Vol. 12.

[22]T. G. Smith, W. B. Marks, G. D. Lange, W. H. Sheriff, Jr., and E. A. Neale, J. Neurosci. Methods **27**, 173–180 (1989).