Retinal Neurons and Vessels Are Not Fractal But Space-Filling

JOSEPH PANICO AND PETER STERLING
Department of Neuroscience, University of Pennsylvania,
Philadelphia, Pennsylvania 19104-6058

ABSTRACT

Many branched patterns in nature are hypothesized to be fractal, i.e., statistically self-similar across a range of scales. We tested this hypothesis on the two-dimensional arbors of retinal neurons and blood vessels. First, we measured fractalness on synthetic fractal and nonfractal patterns. The synthetic fractal patterns exhibited self-similarity over a decade of scale, but the nonfractal “controls” showed hardly any self-similarity. Neuronal and vascular patterns showed no greater self-similarity than the controls. Second, we manipulated a synthetic fractal pattern to remove its self-similarity and found this to be reflected in a loss of measured fractalness. The same manipulation of the nonfractal control and also of the neural and vascular patterns did not alter their measured fractalness. Third, we “grew” patterns of branched line segments according to a variety of nonfractal algorithms. These patterns were, if anything slightly more fractal than the neural and vascular patterns. We conclude that the biological patterns studied here are not fractal. Finally, we measured extended versions of these patterns: a contiguous array of homotypic neuron arbors and a vascular pattern with a high degree of total detail. These patterns showed a “fractal dimension” of 2, which implies that down to some cut-off scale they fill space completely. Thus, neural and vascular patterns might best be described as quasi-regular lattices.

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Historical attempts to characterize visually complex biological patterns such as neuronal arbors have relied on metaphorical descriptions like “bushy,” “stellate,” etc. (e.g., Ramon y Cajal, 1911). Later, quantitative measures of dendritic architecture were added, mostly based upon statistical measures of the length and placement of various dendritic components such as the segments between branch points (Ramon-Moliner, 1962; Scholl, 1967). But these statistical measures offered little insight into the overall structure of the patterns and were not comparable across cells differing in size and branch density. More recently, there have been efforts to develop metrics for classification that are scale-independent; for example, neurons can be classified by differences in “branch length distribution” (Famiglietti, 1992). Most commonly now, neuronal patterns and other biological patterns, such as vascular trees, are characterized by fractal geometry (e.g., Morigiwa et al., 1989; Caserta et al., 1990; Montague and Friedlander, 1991; Takeda et al., 1992; Wingate et al., 1992; Neale et al., 1993; Smith et al., 1993; Kolb et al., 1994; Masters, 1994).

A fractal pattern is “self-similar” in that the pieces of the pattern, when magnified, resemble the whole (Fig. 1A–D). Usually this resemblance means that the part fills space in the same way as the whole, and this is the basis for measuring a pattern’s degree of fractalness. Operationally, a scale-dependent measure of space-filling is chosen, such as the number of boxes in a square grid. The number of boxes intersected by the whole pattern is counted (Fig. 2A), and the procedure is repeated at finer scales (with smaller boxes) as shown in Figure 2B. When the number of boxes intersected at each scale is plotted against box size (on log-log axes), the plot’s linear region indicates that over some range of scale the object is “fractal” (Fig. 2C).

The hypothesis that neuronal and vascular trees are fractal is attractive. If true, it would constrain models of function and suggest models for development. For example, fractal patterns can arise from a simple, diffusion-limited mechanism (Caserta et al., 1992). Also, attempts at classification have been made based on fractal measures (“fractal dimension”; Wingate et al., 1992; Neale et al., 1993; Kolb et al., 1994)). However, there is a serious problem in measuring fractalness of biological patterns, and whether patterns

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Address reprint requests to Peter Sterling, 123 Anatomy-Chemistry Bldg., Department of Neuroscience, University of Pennsylvania, Philadelphia, PA 19104-6058.

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A fractal is "self-similar," meaning that any piece is geometrically similar to the whole. For example, **A** shows the "von Koch triadic curve," an easily constructed fractal shape (see Mandelbrot, 1982). **B** shows that the bracketed piece of curve A, when scaled up, is indistinguishable from the original. An irregular shape can also be fractal. But, unlike the von Koch curve, which is strictly self-similar, an irregular fractal is statistically self-similar. It appears self-similar by some measure that spatially averages the similarity (such as "box counting" in Fig. 2). For example, **C** shows a computer-generated, branched fractal "river" (adapted from Mandelbrot, 1982). The algorithm is carried over 7 iterations. **D** shows that the bracketed piece of curve C, when scaled up, is statistically similar to the original curve; this statistical similarity is demonstrated in Figure 2. Mathematical fractals like A and C can have infinite detail and therefore be fractal over all scales. But natural patterns display limited detail between some high and low cut-off scale. For example, **E** shows an object whose largest scale (high cut-off) is the entire length, while the smallest scale (low cut-off) is the length of one component box. This object displays 1 decade of total scale (size of largest detail/size of smallest detail) = 10/1.

hypothesized to be fractal actually differ from nonfractal patterns has never been tested.

The problem is that while a mathematical fractal like Figure 1A,C can have infinite detail and therefore be fractal over all scales, biological structures have finite detail between some high and low cut-off scale. For an object of finite detail (Fig. 1E), the largest scale (high cut-off) is the largest dimension of the entire shape, while the smallest detail (low cut-off) is the size of the smallest component. The "range of scale" spanned by an object is: (size of largest detail/size of smallest detail), so the object in Figure 1E contains 1 decade of total scale (= 2.3 ln units). Since a pattern can be fractal
Fig. 2. “Box-counting” is one method to measure fractalness. In A the fractal river is overlaid with a grid, and the number of boxes intersected by the pattern counted. In B this is repeated with successively smaller boxes. C plots the (number of boxes intersected) against (box size, i.e., length) on log-log axes. If the log-log plot has a linear region, the pattern is said to be fractal over that range of scale (range of box sizes). Thus, C suggests that the river is fractal over 3.5 natural log units, or roughly 2 decades of scale. But deciding how well the points fit the regression line (for example, by choosing a specific correlation coefficient) was arbitrary. D: To set a more stringent visual criterion, we measured local slopes along the curve and plotted them in a bar graph. The region of true linearity on the curve corresponds to the region of constant local slope. D indicates that the curve in C is linear over only 1 decade of scale (between the arrowheads). E: Fractal river spatially “randomized” by redistributing its component branches randomly in the plane. F: Box-counting method applied to pattern E. The log-log plot in F can still be fit to a regression line with a high correlation coefficient, suggesting that the pattern in E might be fractal. However, see G, G: “Local slopes” plot for graph in F. The local slopes plot exhibits no constant region, demonstrating its greater sensitivity for assessing linearity.
over only the range of scale that it contains, it is difficult to measure fractalness in patterns that span a small range. Here we describe a more sensitive method to identify the “linear region” of the log-log curve. The smaller the segment of the curve considered, the more linear it will be, so this is especially needed for biological patterns because they span a relatively small range of scales (1–2 decades). Next we present an operational test for fractalness: rearranging a pattern on one scale, while leaving it unaltered on a finer scale, should reduce its fractalness. Having established that these tests distinguish fractal from nonfractal, we apply them to biological patterns, including individual neurons and also more extended patterns, such as those formed by an array of neurons.

MATERIALS AND METHODS

Fractal measurement

Box-counting. For this method the pattern was overlaid with a square grid, and the squares (“boxes”) intersected by the line segments were counted (Fig 2A,B). This procedure was repeated with grids of different box size, at intervals of 1.2x, and ln (number of boxes intersected) plotted against the ln (box size Fig 2C). For large boxes the number of boxes intersected is sensitive to the position of the object upon the grid, so we averaged as many as 200 trials for each grid size, randomizing the grid position for each trial.

Sandbox (or mass radius). The sandbox method measures fractalness on pixelated images (Caes et al., 1990) as shown in Figure 3A. A pixel belonging to the structure is chosen as a centering site. Discs of radius \( r \) are centered on it, and for each \( r \) the total number of black pixels (“mass”) falling within the disc is found. This procedure is repeated using each pixel on the structure as a centering site. For each \( r \) the mass found is averaged over all the centering sites used, and the ln of this average mass is plotted against the ln of \( r \) (Fig 3B). To limit the measurement to the pattern’s interior, thereby mitigating “edge effects,” centering sites are chosen only from the interior. The “center of mass” (= the x and y averages of all the Cartesian coordinates on the structure) is taken as the “center” of the pattern. Each point \( p \) in the image has Cartesian coordinates \( p(x,y) \). Then the center of mass (which need not belong to the structure) is \( p_0(x_0,y_0) \), given by \( x_0 = (\sum x)/n \) and \( y_0 = (\sum y)/n \), where \( n \) is the number of points belonging to the structure, and the summation is carried out only over these points. The analogous equation holds for \( y_0 \). The radius of gyration (the average distance from the center of mass over all points on the structure) can be thought of as the radius of the pattern. \( \text{Radius of gyration} = \sqrt{\frac{1}{n} \sum (x_i - x_0)^2 + (y_i - y_0)^2} \). Then the interior of the pattern can be described as all points on the structure that are within \( \text{Radius of gyration} \) of \( p_0(x_0,y_0) \), and centering sites can be limited to this subset of pixels (lightly shaded large disc in Fig 3A).

The sandbox method proved to be less sensitive than box-counting at discriminating fractal from nonfractal among our patterns. However, we evaluate it here because it is one of the most commonly used methods in the literature.

Local slopes. As a more discriminating method for finding the linear region of the log-log plots, we developed the “local slopes” graph (Fig 2D). To construct this graph we calculated a linear regression over a 6-point “window” in the log-log plots, sliding the window along the entire log-log curve. The x axes correspond to the point on the log-log plot around which the window was centered. Using smaller intervals (2 points) for local-slopes calculation increased the “noise” but did not affect the apparent linearity seen in plots.

Pattern representation

All the neurons studied here are quasi-two-dimensional, i.e., their dendritic Arborizations are nearly planar. For instance, in the case of the direction-selective ganglion cell (Fig 8A), the dendritic arbors deviate from the plane by no more than 1 μm, less than 1% of the total arbor extent. Consequently, projecting the pattern onto the plane produces a pattern very similar to the original. This is important since all the fractal measures used here are strictly two-dimensional; i.e., all information about the third dimension was discarded when the pattern was collapsed onto the plane during the original imaging. Our conclusions, as well as all previously reported results (e.g., Fukuda et al., 1988; Caes et al., 1992; Montague and Friedlander, 1991; Takeda et al., 1992; Wingate et al., 1992; Neale et al., 1993; Kolb et al., 1994; Masters, 1994), apply strictly to two-dimensional patterns.

For the “sandbox” method of fractal measurement, pictures of neuronal and vascular patterns were digitized on a 300 dpi scanner to produce black and white images of roughly 500 x 500 pixels. For the “sandbox-counting” method, we used high resolution, black and white images, of roughly 1,200 x 1,200 pixels. These were displayed on a computer monitor and manually reduced to a line segment “skeleton.” A skeleton was represented logically by a collection of line segments, each segment represented by the coordinate pairs of its endpoints.

Synthetic patterns

The synthetic patterns were designed to reproduce the important features of the biological patterns: (i) span a similar range of scale (roughly 2 decades); (ii) branch with roughly the same density (dendritic length/area ratio); (iii) be random rather than strictly regular. The synthetic fractal “river” (Fig 1) was derived from a slight variation on a published algorithm (Mandelbrot, 1982). In the Mandelbrot algorithm, each triangular valley is replaced with a subvalley made of either 1 or 3 subtriangles. In our algorithm, 1 or 2 of the subvalleys may be omitted, as determined by a random number generator. The algorithm was carried over 7 iterations.

We devised a simulator to “grow” patterns as follows: starting at a point, and radiating outward, iteratively add line segments end-to-end. At each iteration add new segments only to the tips of the previous iteration. Each growing tip bifurcates with a fixed probability and fixed branch angle, and can cease growing when another line segment is in its path (self-avoidance).

All of the algorithms used in this paper were written “in-house” in the C and C++ languages, on an IBM RS6000 running AIX and an i486 running Ne XTSTEP for Intel.
Fig. 3. The “sandbox” method measures fractalness on pixelated images (Caserta et al., 1992). **A:** Some pixel belonging to the structure is chosen as a centering site. Discs of radius \( r \) are centered on it, and for each \( r \), the number of black pixels (“mass”) falling within the disc is determined. This procedure is repeated with each pixel on the structure as a centering site. For each \( r \), the mass determined is averaged over all the centering sites, and plotted against \( r \) (In-In) as in B. To limit measurements to the interior of the pattern, and thereby mitigate “border” effects, centering sites are chosen only from the interior. The “center of mass” \( (x \) and \( y \) averages of all Cartesian coordinates on the structure) is taken as the pattern’s center. The “radius of gyration” \( (= \) average distance from the center of mass) can be thought of as the radius of the pattern. Then the pattern’s interior can be described as all points on the structure that are within the radius of gyration of the center of mass. Centering sites can be limited to this subset of pixels. **B:** Sandbox method is applied to patterns from Figures 1, 4, and 5. The decade (2.3 natural log units) of best linearity was found through linear regression, and the slope and correlation coefficient \( (c.c.) \) from that fit is given. The curves are shifted along the \( y \)-axis to avoid overlap: 1C c.c. = .9998, slope = 1.51; 4C c.c. = .9971, slope = 1.77; 4D c.c. = .9992, slope = 1.77; 4E c.c. = .9980, slope = 1.62; 5A c.c. = .9988, slope = 1.77. The biological patterns appear less fractal than the computer-generated fractal river (Figs. 1, 2), and are no more fractal than the nonfractal patterns (Figs. 4E, 5A–C).
Fig. 4. Box-counting applied to five natural patterns (left) and their randomized versions (right). From the log-log plots we generated "local-slopes" plots: A: Alpha ganglion cell (cat; Freed and Sterling, 1988). B: Cultured immature ganglion cell (cat; Montague and Friedlander, 1989). C: "Starburst" amacrine cell (rabbit; Tauchi and Maaland, 1984). D: Angiogram of retina (human; Masters, 1994). E: "Einstein Simplified," after cartoonist Sydney Harris. The biological patterns show no appreciable linear region in the local slopes plot (compare to the truly fractal control in Fig. 2D), nor do the spatially randomizing these patterns reduce their measured fractalness. The biological patterns are not measurably more fractal than the nonfractal cartoon.
RESULTS

Synthetic patterns demonstrably fractal

Our first step was to identify for a synthetic fractal pattern the region of constant local slope along the log-log curve because this more objectively discriminates the linear region. Consider the branched, fractal "river" in Figure 1C. A regression line fitted over almost 2 decades of scale produced a correlation coefficient of 0.9999 and an apparently "good looking" fit (Fig. 2C). The "local slopes" plot showed clearly a constant region, but over less than a decade of scale. Thus, by this criterion the fractal river is fractal over less than a decade of scale. Even though the river is a true fractal and has detail spanning 2 decades of scale, it is measurably fractal over only 1 decade because of measurement imprecision at the scale cut-offs. This is true regardless of which method is used to perform the measurement.

Next, we applied a control manipulation. Since fractalness implies similar placement of components on different scales, randomizing structure on the large scale, while holding it constant on the small scale, should reduce the range over which the pattern is fractal. This proved true for the fractal river; redistributing the interbranchpoint segments randomly (Fig. 2E) removed most of the fractalness (Fig. 2F,G). The small residuum was that contained within the interbranchpoint segments.

Biological patterns not fractal

We applied these tests to two-dimensional arbors of several types of retinal neuron (Fig. 4A-C), including the alpha ganglion cell and a cultured, immature ganglion cell (both from cat), plus a "starburst" amacrine cell (from rabbit). We also tested the pattern of retinal vasculature from primate central retina (Fig. 4D). For all of these patterns, the region of constant local slope spanned no more than half a decade of scale (Fig. 4A-D). This was no greater than for a hand-drawn cartoon (Fig. 4E).

Another fractal measure, the "sandbox" method (Fig. 3) was applied to the original images. This measure proved less accurate than box-counting applied to skeletonized data. For example, with the sandbox method the fractal "river" log-log plot was linear over a substantially smaller range. Thus, by either method, the biological patterns could be considered fractal over at most half a decade of scale.

To determine if the small degree of fractalness in a neuronal or vascular pattern depends at all on the pattern's spatial structure, we applied the second test of fractalness, randomizing the large scale structure. The log-log plots were hardly altered, and the extent of linearity was reduced not at all (Fig. 4A-E). This suggested that the neuronal and vascular patterns might be no more fractal than any other collection of line segments having similar mass and distribution of segment lengths.
Fig. 6. A pattern's "fractal dimension" is the slope of the linear region in the box-counting and sandbox ln-ln plots. It measures how completely the pattern fills space. Thus, the fractal dimension of a line is 1, the fractal dimension of a plane is 2, and the fractal dimension of a two-dimensional pattern that does not fill the plane falls between 1 and 2. For example, B and C are demonstrably fractal patterns with, respectively, $D = 1.5849$ and $1.8928$ (after Mandelbrot, 1982). A is a regular square lattice which completely fills the plane down to the scale of the squares. To appreciate this, consider box-counting on the lattice. When the boxes are larger than the smallest squares in the lattice, all boxes in the counting grid will be hit. Then halving the box size (in the counting grid) quadruples the number of boxes hit (in the counting grid), for a slope of 2 in the ln-ln plots. Thus, $D = 2$.

To test this possibility, we grew arbitrary patterns of branched line segments that spanned the same range of scales as the natural ones. The patterns varied in probability of branching and in distribution of segment lengths, branch angles, and degree of overlap (Fig. 4). However, the algorithm contained no explicit rules that would produce fractalness. Indeed, the "local-slopes" plots for these patterns showed only a narrow constant region, and subsequent randomization had no effect. These diverse artificial patterns were, if anything, more fractal than the natural patterns (compare Fig. 4 with Fig. 5). Thus, despite the common impression that retinal neurons and vessels are fractal, the objective measures applied here show that they are not.

**Biological patterns are space-filling**

We considered an alternative hypothesis, that a neural or vessel pattern actually forms a fairly even mesh with some average pore size, below which there is very little structure. Such a mesh or lattice would actually fill the plane completely down to the scale defined by the pore size (Fig. 6A). If so, then the slope of its log-log plot, (termed the "fractal dimension"; see Fig. 6 legend) ought to be very close to 2. To see why, consider box-counting on a square lattice (Fig. 6A). When the box size equals $x$ (the size of the entire pattern), all four squares of length $x/2$ that comprise it farther down the scale curve will also be intersected. Since halving the box size quadruples the number of boxes hit,
Fig. 7. Box-counting applied to a ganglion cell arbor (from Montagne and Friedlander, 1989) shows that biological patterns tend to be inhomogenous with respect to this measurement. From A to B, boxes are subdivided progressively. Boxes intersected by the neuronal pattern are grey. Where an intersected box is subdivided into four boxes which are also intersected, the fractal dimension is 2. This holds for the interior of the pattern, but not for the boundary where the pattern of box intersection conforms to the outline of the boundary. C: To restrict measurement of the fractal dimension to the pattern’s interior, we used a grid smaller than the total pattern and centered it on the pattern’s center of mass. For the interior, D = 2; for the boundary, D is much closer to 1.

the plot of ln (box size) vs. ln (number of boxes) will have a slope (fractal dimension) of 2. In contrast, box-counting on the structures in Figure 6B and C, which being truly fractal have a range of pore sizes, gives fractal dimensions of 1.58 and 1.89. Now, the fractal dimensions for the neuron and vessel patterns in Figure 4 are all between 1.5 and 1.7. Thus, they appear to fail the test for an even mesh and therefore to resemble fractals.

Yet, the reason why their fractal dimensions are less than 2 is that the structures are spatially inhomogeneous: the borders have a lower fractal dimension than the interiors. This is demonstrated for a neuron in Figure 7. As box size is reduced, all interior boxes continue to be intersected, but along the border some boxes are not intersected. So, over the range of scales shown between Figure 7A and 7B the slope for the interior is 2, but the fractal dimension of the border is less than 2. Most biological patterns contain so little total detail that the measurement of fractal dimension is markedly confounded by this “border effect.” Thus, to measure accurately a pattern’s fractal dimension, measurement must be confined to the interior (Fig. 7C).

Therefore, we analyzed the dendritic meshwork of an array of ganglion cells belonging to a single type (Fig. 8A). The greater extent of the array eliminated the border effect because the interior was large enough (had enough detail) to provide an accurate measurement. Analyzed by both the box-counting and the sandbox method, this meshwork had a fractal dimension of 2 (Fig. 8A). A similar analysis of a
Fig. 8. Fractal analysis of biological patterns when the range of scale is large. A represents not a single neuron arbor, but an array of arbors of directionally selective ganglion cells (Vaney, 1994). B represents a detailed pattern of epifoveal blood vessels (Snodderly et al., 1992). Both include a 5-fold greater range of scale than their counterparts in Figure 4. This provided enough detail/area to restrict the analysis to the pattern interiors, eliminating the contribution from the boundary. For box-counting, the grid was centered on the center of the pattern's bounding box and was limited to roughly 80% its dimension. For the sandbox method, only points within distance 0.7 (radius of gyration) of the center of mass were used as centering sites. For both patterns, box-counting and sandbox methods give a peak fractal dimension of 2. Thus, these neuronal and vascular patterns are "space-filling."
highly detailed pattern of retinal vessels also yielded the same result (Fig. 8B).

**DISCUSSION**

Our measurements on individual neurons and vascular patterns with sparse detail show them not to be fractal. We conclude this because, unlike the control fractal pattern, the retinal patterns showed: (i) no linear region in the log-log plots; (ii) no effect of disarrangement on the log-log plots; or (iii) no difference from any other patterns of branched line segments. This conclusion differs from previous reports on the same structures. The disagreement is not due to any methodological difference because our numerical results were the same. The difference lies rather in the context we have provided for these measurements by introducing the “local slopes” plot and by providing controls: comparable structures with fractal and nonfractal branching.

This negative finding removes the rationale for a class of models for the development of these patterns. Based on the belief that the adult neuronal and vascular patterns are fractal, it has been suggested that their development can be modeled as arising from a diffusion-limited process, such as diffusion-limiting aggregation (“DLA”). Since we have shown that the adult patterns are not fractal, there is no reason to suggest a fractal mechanism for their development. Nor can fractal analysis be useful in classifying neuronal patterns or in measuring their complexity (Winogate et al., 1992; Neale et al., 1993; Kolb et al., 1994). This is because the numerical value of their “fractal dimension” is not independent of scale. Instead, it reflects an average of the pattern’s interior (whose fractal dimension is 2) and its border (whose fractal dimension approaches 1). The numerical values will then depend on the extent of the pattern, and therefore on the size of the arbor.

On the other hand, when measurements of the interior pattern and the border are disentangled, there is a clear positive finding. This was accomplished by measuring larger neural patterns that included many contiguous arbors of the same cell type and vascular pattern that contained considerable detail (Fig. 8). Then the interiors of both patterns had a fractal dimension of 2. We predict that this will be true for other two-dimensional retinal networks such as that of the starburst amacrine cell (Fig. 4C; Tauchi and Masland, 1984), the alpha ganglion cell (Fig. 4A; Wässle et al., 1981), and the delta ganglion cell (Fig. 13, Dacey, 1989). If neurons with extensive arborizations in the third dimension, such as cat beta ganglion cells, are also space-filling, they should have a fractal dimension of 3.

Thus, our present finding that two patterns, one neural and one vascular, fill space, completely offers initial support for a new hypothesis regarding these patterns, namely that they should be regarded as quasi-regular lattices. Such a lattice fills the available space completely down to some cut-off scale represented by the size of its pores; below this there is essentially no structure. Such a lattice guarantees that, on average, no point in the plane is further from some point on the structure that half the mean “pore” size. At the same time, the expenditure of materials and volume occupied by the lattice is minimized. Thus, a quasi-regular lattice would provide an efficient structure for the neural and vascular meshworks to accomplish their biological functions.

From this perspective, a fractal pattern, that by definition contains pores on all scales (Fig. 6B,C), would be inefficient. Large pores in a neuronal meshwork would cause holes in the spatial sampling of synapses from input axons; large pores in a vascular meshwork would correspond to under-perfused areas of tissue. On the other hand, pores finer than necessary would represent redundant structure, a waste of biomaterials, and a waste of critical tissue volume. We conclude that efficiency of “functional architecture” in the adult, rather than the simplicity of developmental rules, is the main determinant of space-filling by these patterns.

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All data, images and skeletons, and all methods, program source code and binaries, for all the analyses in this paper are freely available for anonymous ftp from retina.

anatomy.upenn.edu.

**LITERATURE CITED**


Use and Abuse of Fractal Theory in Neuroscience

J.D. MURRAY
University of Washington, Applied Mathematics, Seattle, Washington 98195

The problem with a good name for a new field is that uninformed proselytising and inappropriate use can raise unrealistic expectations as to its relevance and applicability. This is particularly true for fractal theory which can be visually dramatic and can be practised without much background or sophistication. Catastrophe theory is a further example: its mathematical practitioners did considerable harm to the cause of interdisciplinary biological-mathematical research. Although chaos and fractal theories have been proposed by some as biological panaceas, fortunately there are enough realists to counter this view and keep them in perspective. Chaos theory, and what has been referred to as a "new mathematics," namely nonlinear theory, are considerably more relevant in many biological situations.

A particular and widespread misconception about fractal theory arises because it can create objects which look remarkably like many natural structures such as trees, weeds, flowers, butterfly wing patterns and so on, and this is often taken to be a biological explanation of how these structures and patterns are formed. Although fractal-like patterns may be reasonable graphical representations of such natural shapes, they say essentially nothing about the biological processes and mechanisms which are involved in their development. Considerably more is required of a model.

Perhaps we should keep in mind what a truly scientific model must try to do. It is essential to: (i) start with the real biological situation and try to isolate the key steps in a process, (ii) try to construct a model mechanism which reflects these key elements and involves real biological quantities, (iii) investigate the theoretical model mathematically and obtain solutions with biologically realistic boundary and initial conditions and, most importantly, (iv) on the basis of the theoretical results, return to the biology with predictions, comments and suggestions for illuminating experiments which will help elucidate the underlying mechanisms. If the results do not agree with the known biology, the circular process must be repeated. The most notable successes are those in which the experimentalist and theoretician work on the model and interpretation together. There are many such illustrative modelling examples (Murray, 1989) where both the biology, models and subsequent experiments are described in detail. Other examples, where the modelling dictated subsequent experiments, are described in Oster et al. (1988) and Murray et al. (1990).

In view of the sometimes uncritical use of fractal theory, the paper by Panico and Sterling (1995) is particularly relevant. They are concerned with branching patterns in a variety of neuronal cells. In a very systematic and convincing way, they suggest that a reason for the specific branching patterns is much more straightforward than has been suggested by a fractal approach, which measures "fractal dimension" with a view to classification. They show that such cells are in fact space-filling rather than fractal. This is a much more down to earth motivation if a branching structure is trying to maximise such things as spatial coverage without redundancy. A simple everyday example such as that illustrated in Figure 1 makes the point. There are methods for evaluating the fractal dimension of branching structures, such as the box counting method (see, for example, Bassingthwaighte et al., 1994), which, if applied to the winter branch structure (Fig. 1b) of the ivy on the wall, would give a fractal dimension between 1 and 2. This, it seems to me, says nothing about the actual biological problems of interest. Looking at the branching structure and the subsequent summer foliage, the fact that the branching structure is space-filling, as it almost certainly is trying to do, is much more revealing. It immediately poses more interesting questions as to how the branching is actually affected and how interbranch space is detected. Although some of the concepts of fractal geometry can certainly be useful, the article by Panico and Sterling (1995) introduces a very necessary note of realism into what could become a deluge of irrelevant uses of fractal measurements.

One genuine practical application of fractal theory is directly related to the measurement of biological structures at different magnifications. We can think of fractals in a simplistic, but still useful way as geometric figures which repeat themselves at progressively smaller scales or exhibit progressively more complex structure when observed at larger magnifications. With a fractal there is often self-symmetry, or approximate self-symmetry. That is, if we magnify a small part of the overall pattern it more or less displays some aspects of the whole pattern. There are now many books on the subject; these often include discussions of chaos, which is a closely related subject. Most of the books present dramatic figures similar to many observed in nature, some dwell on the interesting mathematical theory, but singularly few deal with pattern formation processes at the level we are interested in. The work of Bassingthwaighte et al. (1994) is specifically devoted to physiological problems. They provide basic background material on fractals (and chaos) and apply it to specific physiological processes. They also point out that similarity in pattern says nothing about the mechanisms which produce them. The work of Falconer (1990) also provides a clear introduction to the theory and applications of fractals generally.

Fractal theory is being used in quite diverse fields and it is proving to be an increasingly useful tool. It can, however, be a very blunt instrument. For example, it is possible to

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Fig. 1. Ivy-covered wall in winter and summer. Is the branching structure in (b) fractal or is it space-filling to optimise the leaf coverage (a) in the summer?

Fig. 2. Typical pattern generated by diffusion-limited aggregation. (From Bassingthwaighte et al., 1994 and reproduced with permission.) Although there is a visual similarity with typical amacrine cells from the central region of the rabbit retina, it is premature to draw any conclusions as to the mechanism which forms the cellular patterns solely by visual comparison between it and the diffusion-limited pattern.
have two quite different branching structures which have the same fractal dimension. Something much more is obviously needed to distinguish the two types. Ideally, this should be information about the mechanism involved in their formation. It has also frequently been suggested that if a pattern is fractal, then we can infer something about the mechanism which generates it. The case most frequently cited is when the pattern generated looks qualitatively similar to that in Figure 2 which is a typical pattern generated by diffusion limited aggregation. Diffusion limited aggregation (DLA) is a diffusion process whereby particles exhibit a random walk behaviour, and when a particle comes into contact with another particle it sticks to it and can no longer move. The process is usually started with a seed of stationary particles onto which released particles eventually diffuse and accrete. In this way, a spatial pattern is dynamically formed which is fractal in character. In this DLA example, the theoretically computed fractal dimension is 1.7. We can also use the box dimension method, for example, to compute it from the final figure. If the reader now looks at some of the cell types in the article by Panico and Sterling (1995), there is a certain gross similarity between Figure 2 and the amacrine cell from the central region of the rabbit retina. Although it is doubtful that diffusion limited aggregation has anything to do with the pattern formation mechanism which generates the cellular pattern, it has been suggested (for example, by Caserta et al., 1990 and Schierwagen, 1990) that the shape of neuronal cells may be determined by this process. This is an unjustified conclusion to draw from mere visual similarity. Any model mechanism, such as diffusion-limited aggregation, must be judged against other biological spatial patterning generators and by the experiments each of them suggest. It is only by these means that they can be differentiated. In the light of the easy generation of hypothetical ferns, trees and so on by fractal generators, it is easy to forget the main purpose of studying pattern formation in biology, namely to discover the underlying biological processes which produce the spatial patterns.

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LITERATURE CITED


