

Fractality of dendritic arborization of spinal cord neurons

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Abstract

Skeletonized images of Golgi impregnated neurons from the human, monkey, cat and rat dorsal horns were subjected to fractal analysis. These neurons have sparse branching of dendrite arbors. It is noticed that, in certain neuronal samples, some authors report that scaling range of experimentally declared fractals is extremely limited and spanned approximately between 0.5 and 2.0 decades. In order to retain our hypothesis that neurons with dendrites of uncomplicated shapes can be considered fractal over three decades of scale, we conducted four procedures: (i) we used the box-counting method, (ii) we scaled the box sizes as a power of 2, (iii) we chose the coefficient of correlation, measuring the “goodness of fit” of experimental data points to regression straight line, to be equal to or larger than 0.995, and (iv) we pointed out that all the neurons analyzed have a single fractal dimension measuring a global fractality showing no linear regions. As a control, we used some cerebellar Purkinje cells whose dendrite trees show much more complex structure and profuseness of branching. Since, generally, the neuronal structure is among the most complex of all cellular morphologies, we believe that supporting this hypothesis we advance the neuroscience and fractal theory.

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Fractal geometry, as a contemporary branch of pure and applied mathematics and the basis of all fractal ideas, is developed as a new geometry of nature [11]. Mandelbrot established this geometry to describe the complexity of forms and processes found in nature [1,11]. In fractal geometry, a fractal should be comprehended as a basic (most general) concept, that is, as a theoretical abstraction that cannot be defined analytically. Two types of fractals are being used in quite different fields: mathematical (geometric) and statistical (natural, empirical) fractals. Mathematical fractals are mathematical constructions characterized by never-ending cascades of similar structural details [11]. Natural fractals are more restricted than are mathematical ones. Fractal objects in space possess two important properties: self-similarity and scaling. An object is geometrically self-similar if its pieces are exact duplication of the whole object [11]. On the other hand, if a property is measured on a piece of a naturally occurring object at high resolution, the object is said to be statistically self-similar if the small portions of the object, when magnified, resemble qualitatively the whole. This resemblance means that, for instance, the part fills space in the same way as the whole. The pieces of natural objects are rarely exact reduced copies of the

whole object. Statistical self-similarity implies that the property being measured depends on the size (or resolution) of the scale used to do the measurement. Thus the property scales with the measurement used to measure it. For instance, a scale-dependent measure of a space-filling object can be chosen as the number of boxes in a square grid. The number of boxes intersected by the whole object is counted and the procedure is repeated at finer scales (smaller boxes). The self-similarity and scaling can be quantitatively estimated by the fractal dimension D . This measure describes the complexity of form and space-filling property of an object.

The rule showing how the measured value depends on the resolution of measurement is called the scaling relationship. The simplest scaling relationship determined by self-similarity of an object is given by power law scaling [1]

$$P(r) = Br^{\alpha(D)} \quad (1)$$

where P is a measured property of the object (such as, length, area, or volume), B a factor for the power law scaling, r the scale (resolution) at which it is measured, and $\alpha(D)$ is the scaling exponent, representing a simple function of the fractal dimension D of the object. This function depends of the Euclidean dimension E of the object embedded in space and is accepted to be $\alpha(D) = E - D$. Taking the logarithms of both sides of Eq. (1)

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yields

$$\log P(r) = \alpha(D) \log r + \log B \quad (2)$$

Thus, power law scaling is revealed as a straight line when the logarithm of the measured property P is plotted against the logarithm of the scale r at which it is measured.

While a mathematical fractal requires infinite orders of magnitude of power-law scaling and therefore is fractal over all scales, physical, biological and other structures in nature have a finite number of decades between a high and a low cut-off scale. The scaling ranges of experimentally declared fractals are limited, often to five decades or more [11]. Neuroscience shows an even more negative situation. Biological patterns span over a relatively small scaling range, mainly between 0.5 and 2 decades. Panico and Sterling [13] claimed that these patterns could be considered fractals over at most half a decade of a scale. Caserta et al. [3] considered that any natural fractal should be fractal over at least one decade. Smith et al. [17] found that the slope of the regression straight line is constant over about two decades of magnitude. Takeda et al. [19] reported that obtained regression lines show a very good fit over less than one decade. Fernández and Jelinek [5] pointed out that the actual data points are generally not laid over a straight line for more than one or two decades. They concluded that biological data having a linear fit of more than two orders of magnitude is extremely rare. Such short intervals of linearity in natural fractals represent additional difficulties in determining linearity and fractal character.

The range of the linear slope of the log–log plots indicates the range of self-similarity [17,18]. If the log–log plot has a linear region, the pattern is said to be fractal only over that range of scale [13]. In all practical measurements the issue to be addressed is the “goodness” of straight line fit and is usually decided according to some statistical criteria (e.g., by the coefficient of correlation between the data and a straight line model based on, for instance, the least-square method) [18]. But deciding how well the regression line fits the points was arbitrary [13]. For instance, Takeda et al. [19] used $R > 0.999$ for such criterion, Jelinek and Fernández [9] used $R > 0.998$. We have used the value 0.995 [12]. A discriminating method for finding the linear region of the log–log plots is rarely proposed [13]. Considering everything, it has been adopted by most of the authors that this range is between 0.990 and 0.999.

The aim of the present report is to show that using suitable neurological material, corresponding procedure of measurement and image processing, the interval of self-similarity of the neurobiological patterns can be extended over three orders of magnitude.

The images of Golgi impregnated neurons of human and rat spinal cords were obtained as reported in our previously published article [12], and those of monkeys and cats were based entirely upon the experimental data published in two original articles [2,6]. We have also used as a control six Purkinje cells in the cerebellum of mouse from two publications [17,19]. These cells have highly branched dendrites. All neurons were used from adult specimens and pooled into four groups as shown in Table 1.

Table 1

A summary of the 114 cells used in the experiments

Species	N^0	R		%
		≥ 0.995	< 0.995	
Monkey	17	14	3	21.4
Cat	24	24	0	0
Human	30	28	2	7.1
Rat	43	42	1	2.4
Total	114	108	6	5.6

Methods of image preparations and fractal analysis are outlined in the mentioned study [12] and are not covered here. Briefly, the camera lucida drawings were digitized by a scanner with maximal resolution (600 dpi) in order to generate authentic digital images of the drawings. In their later work, Jelinek et al. [8] have analyzed the fractal dimension at different resolutions of a neuronal image. They concluded that cells scanned at low resolution had higher values of fractal dimension than those obtained at high resolution. Analyzing our experimental material we have drawn the same conclusion. Since our topic is the influence of dendrite complexity to the fractal character of neurons, we have scanned all our drawings using maximal resolution to get digital images that would maximize the resemblance between the digital and original drawings. It seems likely that such images retain most of the neuronal details, enabling reduction of experimental errors in the fractal measurements.

All the drawings were analyzed as skeleton images using the public domain Image J software. All scanned images were imported into the software. Axons, spines and soma were removed digitally. Then the program performed a skeleton of the image to a stick figure. The images were analyzed by the box-counting method using the same software. Basically, this method consists of “covering” the image border with sets of squares. Each set is characterized by the size r of the square edge. The corresponding number of squares $N(r)$ necessary to cover the border is presented as a function of r . The fractal dimension D is determined from the slope S of the log–log relationship between $N(r)$ and r , as $D = 1 - S$ [18]. The interval of box sizes was taken from 2 to 1024 pixels (when possible, it was taken from 1 to 2048 pixels). We adopted the coefficient of correlation R to evaluate the “goodness of fit” of the regression line to data points valued 0.995 or higher.

In order to retain our hypothesis that the cells with dendrites of uncomplicated shapes and sparse branching patterns (Fig. 1A) could be considered fractal over three decades of scale, we used, as a control, few cells (cerebella Purkinje cells in mouse) whose dendrite trees show more complex structures and profuseness of branching (Fig. 1B), and apply some techniques and image preparations which seem to be useful to achieve it.

- (i) When performing fractal analysis it should be noted that different methods work best for different fractals. To test our data for fractal character, we have used the box-counting method which is both popular and commonly used for

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