Fractals, Vol. 2, No. 1 (1994) 95–102

DENDRITIC COMPLEXITY AND THE EVOLUTION OF CEREBELLAR PURKINJE CELLS

B. R. KRAUSS, B. J. SEROG, D. R. CHIALVO and A. V. APKARIAN*
Dept., Neurosurgery, Computational Neuroscience,
SUNY Health Science Center at Syracuse, NY

Received June 25, 1993; Accepted December 1, 1993

Abstract

Cerebellar Purkinje cells have the most extensive dendritic arborization in the central nervous system. These neurons can be readily identified in most vertebrates. We reexamined a recently postulated relationship between phylogeny and fractal dimension of Purkinje cells' dendritic field (Takeda et al.[1]). The fractal dimension of Purkinje cells was measured, using a box-counting technique, from several species with diverging eras spanning 5.24×10^8 years. The linear correlation ($y = mx + b$) between phylogenetic placement and fractal dimension, for water creatures gave $m = -0.057$, $r^2 = 0.137$ and for land creatures $m = -0.096$, $r^2 = 0.685$. Regression analysis showed that the slope for the water creatures line was not significantly different from zero, but the slope for the land creatures was significant. These results only partially agree with the conclusions of Takeda et al. We also found that small variations in data collection and analysis can significantly change these results, including: quality and technique of cell reconstruction, criteria of selection of the scaling region, and tolerance on the power-law fit.

*E-mail: apkarian@vax.cs.hscsyr.edu
Send correspondence to:
A. Vania Apkarian
SUNY Health Science Center, 766 Irving Ave, WH 3118, Neurosurgery Research Labs, Syracuse, NY 13210.

1. INTRODUCTION

The cerebellar cortex consists of three cell layers that contain five different types of neurons: stellate, basket, Purkinje, Golgi and granule cells. Purkinje cells have the most extensively branched dendrites which are confined to a single plane, perpendicular to the main axis of the folium. Purkinje cells in the cerebellum are readily identifiable in most land and sea vertebrates, and their morphology has been extensively studied by many anatomists. Therefore, Purkinje cells are ideal for studying the relationship between evolution and the complexity of dendritic arborization.

The complexity of the Purkinje cells' dendritic fields can be quantified by measuring their fractal dimension (D_f). Fractal geometry, first introduced by Mandelbrot,[2] provides a basis for the analysis and interpretation of complex forms found in nature. It is now widely recognized that objects such as the snail shell, clouds, the bronchial tree, and mountains cannot be adequately described by Euclidean geometry. The invariance of these shapes over different scales, expressing statistical self-similarity, is well quantified by the fractal dimension. This value is a noninteger number, which indicates the space filling properties of the object. the fractional portion of D_f reflects the extent of space filling and, thus, it can also be regarded as a measure of the complexity of the structure.

Mandelbrot[2] has suggested (page 162) that perhaps the Purkinje cell exhibits a decrease in complexity in older species, which might be quantified by the fractal dimension. He said, "It would be nice if this [referring to phylogenetic developmental time] corresponded to a decrease in D, but the notion that neurons are fractals remains conjectural." The fractal properties of neurons have now been studied by a number of groups.[3–9] The results indicate that neurons exhibit statistical self-similarity over a limited range of scales. Some have also speculated that Purkinje cells may also exhibit deterministic self-similarity.[10] Others have been unable to find large enough size scales where neurons exhibit self-similarity and thus have questioned whether neurons can be considered fractal objects.[11] Takeda et al.[1] studied Purkinje cells from land and sea creatures that span 5.24×10^8 years and concluded that there is a link between evolution and complexity measured by the fractal dimension. Independent of Takeda et al.'s work, we were pursuing a similar investigation. Following publication of their work, we incorporated their data and our own and reexamined the relation between phylogeny and Purkinje cell dendritic complexity. Here, we report only a partial agreement with Takeda et al.'s conclusions. We also point to a number of difficulties associated with algorithms used for estimating D_f, and propose techniques to overcome these problems.

2. METHODS

The fractal dimensions of Purkinje cells were determined from photocopies of camera-lucida drawn golgi-impregnated cells. Digitized video images of human Purkinje cells were also used. The video images were captured by a video camera mounted on a microscope and connected to a personal computer (R&M Biometrics). The images were scanned using an HP Scanjet Plus and a personal computer used for calculations. A box-counting method[2] was used to estimate the fractal dimension. The algorithm initially encloses the structure under study with a single square box. This box is then dissected into smaller constituent boxes and the number of boxes filled with the structure counted. The log-log plot of the number of boxes covering the structure vs. the box length indicates the scaling properties of the structure. The slope of the regression line through the data estimates D_f. Since biological structures have a limited size, the range of sizes within which the regression line is determined is critical in calculating D_f. The box counting algorithm was repeated three times for each neuron, each time the neuron being rotated by about 90°, and D_f was calculated. The mean and standard deviation of D_F was thus determined for each cell.

Some of the data of Takeda et al. were used. The cells with the best resolution were selected from the data that Takeda et al. used. Single cells for lamprey, shark, perch, frog, turtle, and pigeon were taken from Nieuwenhuys's chapter,[12] which are reproductions of Golgi impregnated Purkinje cells from various anatomists. The mouse cell is from Fig. 1 of Takeda et al.[1] The human Purkinje cell was taken from Cajal.[13] In addition Purkinje cells for the ray, *Gnathonemus petersi*, and chicken were taken from Llinas.[14] The rat Purkinje cell was taken from Barry and Bradley.[15] Also, Golgi impregnated human slides were supplied by the Anatomy Department of SUNY Health Science Center at Syracuse. The latter slides were used to analyze the

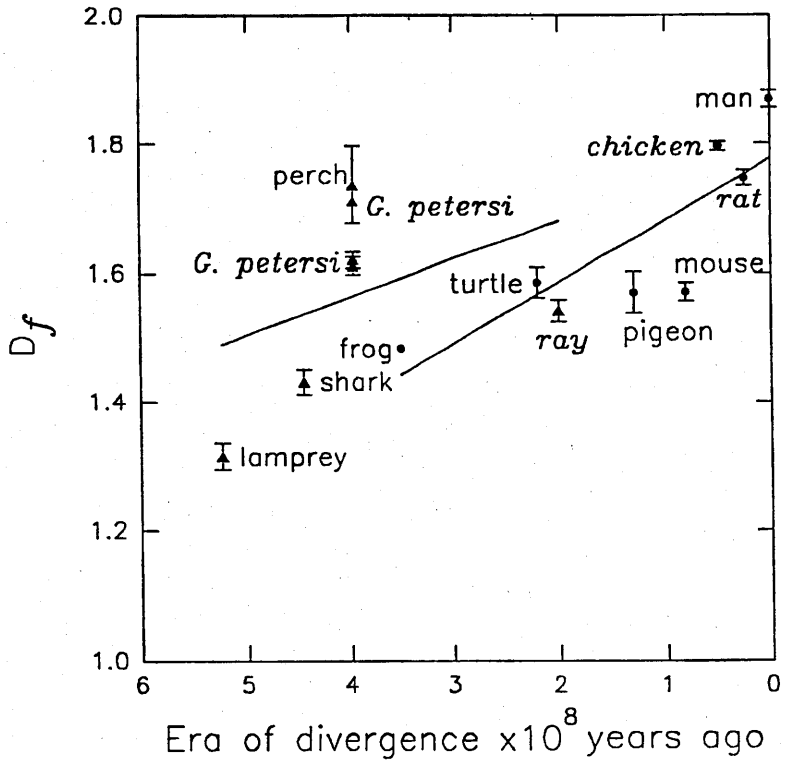


Fig. 1 Fractal dimension (D_f) of Purkinje cells as a function of diverging era for water (triangles) and land (circles) species. Species in italics do not appear in the data from Takeda et al. Vertical bars represent one standard deviation. The frog standard deviation cannot be displayed since it is too small. The least squares fit lines for water and land creatures are shown separately.

cells using video capturing. All the other cells were analyzed by scanning the enlarged published figures. We used the same divergence eras for the species used by Takeda et al.[1] For chicken, rat and ray, divergence eras were taken from Carroll.[16]

3. RESULTS

The dendritic complexity of the various species studied both by us and by Takeda et al. is presented in Table 1. The linear correlation between phylogenetic place and D_f was calculated for the water and land creatures. The correlation ($y = mx + b$) between diverging era (in years $\times 10^8$) and D_f for water creatures was $m = -0.057$, $b = 1.792$, $r^2 = 0.137$, and for land creatures $m = -0.096$, $b = 1.779$, $r^2 = 0.685$ (Fig. 1). Regression analysis showed that the slope of the water creatures line was not significantly different from zero ($F = 0.8$, $p = 0.41$), but the slope of the land creatures was ($F = 10.89$, $p = 0.02$). In contrast, the slopes of the previously reported results of Takeda et al. were: water creatures, $m = -0.477$, $r^2 = 0.998$ and land creatures, $m = -0.113$, $r^2 = 0.991$. Both our analysis and Takeda et al.'s show a tendency for the fractal dimensions of the species examined to increase as the era of divergence decreased. However, our results show larger scatter between species and no statistical significance for the water creatures.

The fractal dimensions obtained were, in most instances, different between the two investigators, even though the neurons and the general methods used to calculate the fractal dimensions were the same (Table 1). This indicated that small variations in data collection techniques and analysis result in different estimates of D_f. This idea was tested by calculating the D_f of a Purkinje cell of a 15-day-old mouse taken from Fig. 1(a) of Takeda et al.[1] Figure 2 is a log-log plot of the number of boxes vs. box length for this mouse data. The least squares fit method was applied to points 18–89 in this cell, giving a slope (D_f) of 1.65. $r^2 = 0.99905$. For this same neuron, Takeda et al., after performing the least squares fit on the same points 18–89, report a D_f of 1.71, $r^2 = 0.99991$. This example

Table 1 Fractal Dimension of Purkinje Cells

	Krauss et al.		Takeda et al.	
Species	**Fractal dimension**	**# Rotations**	**Fractal dimension**	**# Cells**
Lamprey	1.31 + / − 0.02	3	1.13	1
Shark	1.43 + / − 0.02	3	1.48	1
Perch	1.74 + / − 0.06	3	1.74	1
Frog	1.48 + / − 0.002	3	1.45 + / − 0.13	3
Turtle	1.58 + / − 0.02	3	1.65 + / − 0.12	2
Pigeon	1.57 + / − 0.03	3	1.72 + / − 0.04	2
Mouse	1.57 + / − 0.02	3	1.68 + / − 0.04	4
Human	1.87 + / − 0.01	3	1.86 + / − 0.03	3

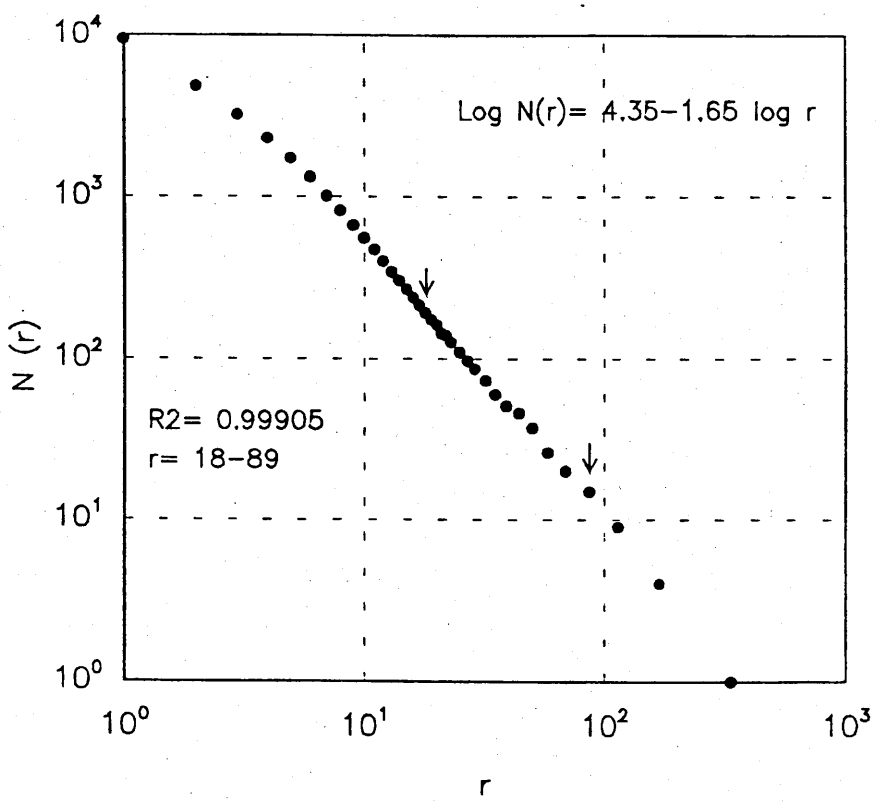


Fig. 2 The scaling properties of a mouse Purkinje cell. A log-log plot of the number of boxes, $N(r)$, covering the dendritic field as a function of box size, r. The least squares fit was applied to points 18–89 in this cell giving a slope, D_f, of 1.65. Arrows indicate the range of points used for the least squares fit. This neuron is Fig. 1(a) of Ref. 1.

shows that even small differences in data collection and analysis result in different fractal dimensions.

An alternative to scanning camera-lucida drawn cells is video capture of golgi-impregnated cells directly from the slides. Four human Purkinje cells were analyzed by this technique, two of which are shown in Fig. 3. Unretouched [Figs. 3(a) and 3(c)] and sharpened [thresholded, Figs. 3(b) and 3(d)]

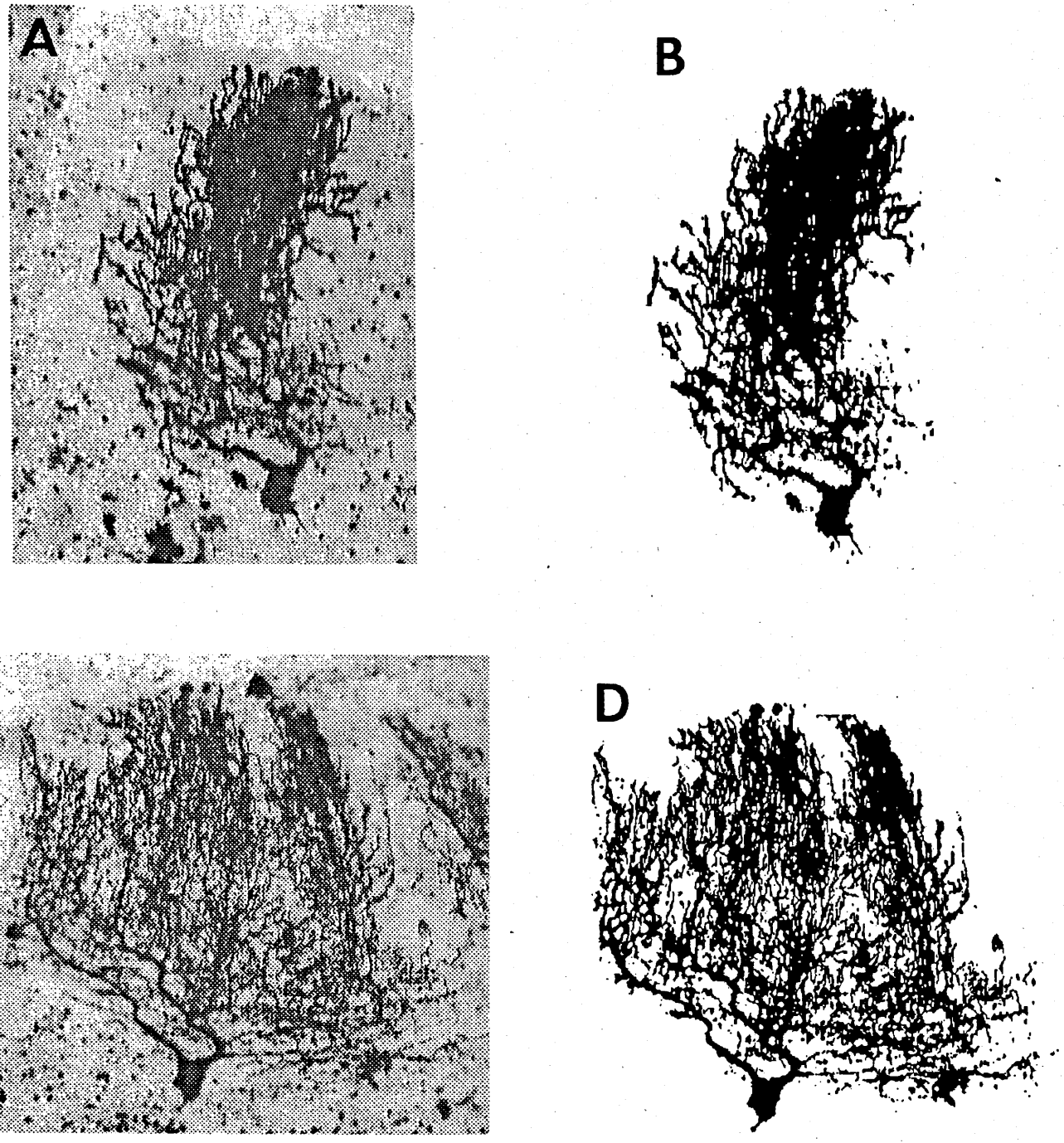
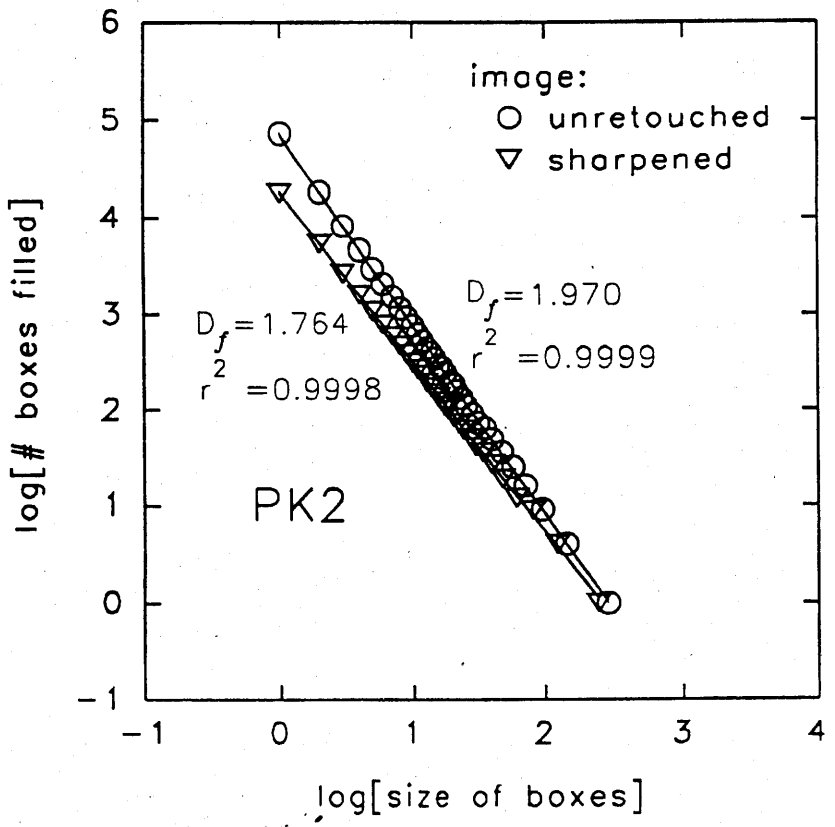


Fig. 3 Two human Golgi-impregnated Purkinje cells video captured directly from tissue slides. **(a and c)** Unretouched pictures. **(b and d)** The same two cells as in (a) and (c) after sharpening (thresholding), applied to decrease the background. The cell in (a) and (b) is PK2 in Fig. 4 and the cell in (c) and (d) is PK4 in Fig. 4.

images were used in box-counting for both cells (Fig. 4). The unretouched images result in a higher estimate of D_f, 1.951 and 1.970, $r^2 = 0.99982$, $r^2 = 0.99993$, respectively. The D_f of the sharpened images were 1.764 and 1.808, $r^2 = 0.99979$, $r^2 = 0.99977$, respectively (Fig. 4). The self-similarity of these video-captured images is maintained over two decades. In comparison, the self-similarity of the hand drawn images analyzed by us and by Takeda et al. were limited to one decade. The mean D_f was 1.750, for the four human Purkinje cells with a standard deviation of 0.047 ($n = 4$). This estimate



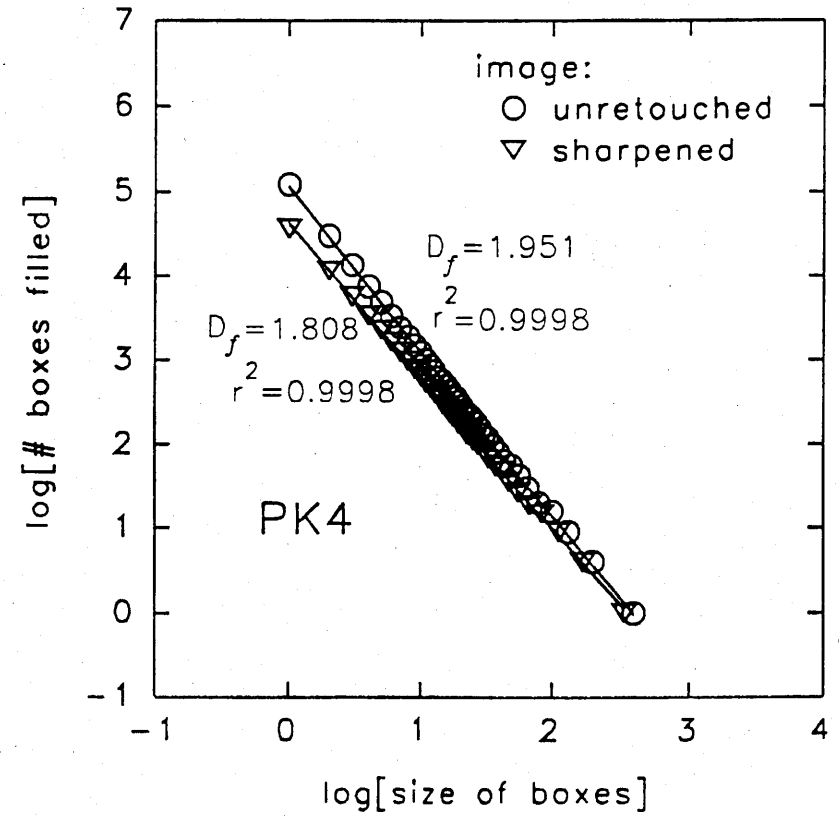


Fig. 4 Scaling properties for the two cells shown in Fig. 3 (PK2 and PK4). Log-log plots comparing unretouched (circles) and sharpened (triangles) images of the cells. The D_f and correlation coefficient (r^2) are shown for each case.

of D_f for human Purkinje cells is significantly different from that reported by Takeda et al. (t-test, $p = 0.017$).

4. DISCUSSION

With the species selected, there was a tendency for fractal dimensions of Purkinje cells to increase over evolutionary time. This tendency was statistically nonsignificant for the water creatures, and significant for the land creatures. Takeda et al. show a tighter correlation between the estimated D_f and diverging era for the 3 water species and 5 land species they examined. Our correlation with 12 species is much looser. In the sea species, our results would very closely agree with Takeda et al.'s if we eliminate the ray. For the animals studied by us and by Takeda et al., in some of the species we estimated a higher D_f (lamprey) and in other species a lower D_f (mouse) than Takeda et al. Therefore, there are no obvious systematic differences between our results and that of Takeda et al. The variance of the regression lines for water and land species seems to increase with the increasing number of species. This observation implies that there are other variables, besides evolutionary scale, that need to be taken into consideration to explain the Purkinje cell complexity. In the land creatures, perhaps different regression lines for amphibians, reptiles, birds and primates would be more meaningful. More importantly, the presumed function of the neurons under study must be taken into consideration. For example, assuming that Purkinje cells have something to do with motor learning, a scale of the motor abilities of the species may explain away a large amount of the variance.

Data collection methods can change the fractal dimension estimate of a neuron. To introduce the least amount of variation, neurons should be reconstructed by the same individual using the same scale. Most of the data used by us and by Takeda et al. were taken from published literature. Since these data are camera lucida drawings by different individuals, the conclusions reached by us and by Takeda et al. should be viewed cautiously. Video capture of stained cells, which eliminates the above-mentioned biases is an alternative to camera-lucida drawn cells. Most importantly, video capture eliminates subjective judgments that are endemic in camera lucida reconstructions. Video capturing has the inevitable disadvantage of including a certain amount of background in the analysis. The degree of this contamination can be reproducibly controlled by defining a fixed threshold for all images. It is important to note that the video captured Purkinje cells displayed a much larger range of self-similarity than the camera lucida reconstructed cells. Presumably, this is a reflection of the amount of detail that is ignored during camera lucida reconstructions. Therefore, we recommend the video capture approach over the camera lucida reconstruction of neurons for estimation of fractal dimension, whenever possible.

ACKNOWLEDGMENT

We wish to thank B. B. Mandelbrot, who suggested to us to look at the fractal dimensions of Purkinje cells. We also thank the Anatomy Department at SUNY Health Science Center at Syracuse for lending us Golgi stained tissue. This project was funded by the Neurosurgery Department at SUNY Health Science Center at Syracuse.

REFERENCES

1. T. Takeda, A. Ishikawa, K. Ohtomo, Y. Kobayashi and T. Matsuoka, "Fractal Dimension of Dendritic Tree of Cerebellar Purkinje Cell During Onto- and Phylogenetic Development," *Neurosci. Res.* **13**, 19–31 (1992).
2. B. B. Mandelbrot, *The Fractal Geometry of Nature* (Freeman, Oxford, 1983).
3. F. Caserta, H. E. Stanley, W. D. Eldred, G. Daccord, R. E. Hausman and J. Nittmann, "Physical Mechanisms Underlying Neurite Outgrowth: A Quantitative Analysis of Neuronal Shape," *Phys. Rev. Lett.* **64**, 95–98 (1990).
4. F. Caserta, G. Hausman, W. D. Eldred, C. Kimmel and H. E. Stanley, "Effect of Viscosity on Neurite Outgrowth and Fractal Dimension," *Neurosci. Lett.* **136**, 198–202 (1992).
5. A. K. Schierwagen, "Growth, Structure and Dynamics of Real Neurons: Model Studies and Experimental Results," *Biomed. Biochim. Acta.* **49**, 709–722 (1990).
6. E. A. Neale, L. M. Bowers and T. G. Smith, Jr., "Early Dendrite Development in Spinal Cord Cell Cultures: A Quantitative Study," *J. Neurosci. Res.* **34**, 54–66 (1993).
7. R. J. T. Wingate, T. Fitzgibbon and I. D. Thompson, "Lucifer Yellow, Retrograde Tracers, and Fractal Analysis Characterize Adult Ferret Retinal Ganglion Cells," *J. Comp. Neurol.* **323**, 449–474 (1992).

8. R. Porter, S. Ghosh, G. D. Lange and T. G. Smith, Jr., "A Fractal Analysis of Pyramidal Neurons in Mammalian Motor Cortex," *Neurosci. Lett.* **130**, 112–116 (1991).
9. K.-D. Kniffki, D. Chialvo, C. Vahle-Hinz and A. V. Apkarian, "Fractal Dimensions of Neurons Located in the Cat's Thalamic Ventrobasal Complex (VB) and Its Ventral Periphery (VB_{vp})," *Soc. Neurosci. Abstr.* **17**, 622 (1991).
10. A. J. Pellionisz, "Neural Geometry: Towards a Fractal Model of Neurons," in *Models of Brain Function*, ed. R. M. J. Cotterill (Cambridge University Press, New York, 1989), pp. 453–464.
11. J. Panico and P. Sterling, "Retinal Ganglion Cells are not Fractal in the Box Counting Measure," *Soc. Neurosci. Abstr.* **18**, 1030 (1992).
12. R. Nieuwenhuys, "Comparative Anatomy of the Cerebellum," in *Prog. Brain Res. Vol. 25, The Cerebellum*, eds. Fox and Snider (Elsevier, Amsterdam, 1967), pp. 1–93.
13. S. Ramon y Cajal, *Histologie du systeme nerveux de l'homme et des vertebres*, Vols. I & II (1911).
14. R. Llinas, *Neurobiology of Cerebellar Evolution and Development* (American Medical Assoc, Chicago, 1969).
15. M. Berry and P. Bradely, "The Growth of the Dendritic Trees of Purkinje Cells in the Cerebellum of the Rat," *Brain Res.* **112**, 1–35 (1976).
16. R. L. Carroll, *Vertebrate Paleontology and Evolution* (W. H. Freeman & Company, New York, 1988), pp. 77–350.