## Comment on "Physical Mechanisms Underlying Neurite Outgrowth: A Quantitative Analysis of Neuronal Shape"

A recent Letter by Caserta *et al.*[1] reported on measurements of the dendritic outgrowth from mammalian retinal ganglion neurons grown both *in vivo* and *in vitro*. This work showed that the extent of the outgrowth was well described by a power law and thus was amenable to analysis in terms of a fractal dimension. Neurons in the adult animal were found to have a dimension of $d_f = 1.69 \pm 0.15$, while cells that were dissociated from embryonic animals and allowed to develop in culture had an average dimension of $d_f = 1.4 \pm 0.1$. Caserta *et al.*[1] suggest that, at least *in vivo*, the outgrowth can be considered in terms of a diffusion-limited-aggregation (DLA) process in two Eucledian dimensions, for which $d_f = 1.7 \pm 0.1$.[2]

An important question raised by this[1] and related[3,4] work is whether the difference in dimension for neurons *in vivo* versus *in vitro* represents a difference in the developmental age of the animal or is an artifact of culture conditions. One difficulty in answering this question is that adult mammalian central neurons cannot be maintained *in vitro*.[5] In contrast, neurons from adult invertebrates can be maintained *in vitro*.[6] These cells regenerate extensive neurites in culture, suggesting their utility in resolving this question.

Here we report on observations of the outgrowth from *Aplysia* neurons *in vitro*. Cell bodies of metacerebral neurons were isolated from adult animals and allowed to regenerate in culture, as described.[7] The neurites cross over each other, but the outgrowth was essentially planar. Silhouette drawings of the cells were prepared after 4 d *in vitro* (Fig. 1, inset) and digitized for analysis. The fractal dimension was computed using the box method.[1] We counted the fractional area covered by the outgrowth for a series of apertures of increasing radius. The area occupied by the cell body was excluded from this count.

The results for a typical neuron are shown in Fig. 1. We plot the logarithm of the area of the aperture filled by cellular outgrowth, equivalent to the cluster mass $M(r)$ of Ref. 1, versus the logarithm of the total area of the aperture, $A = \pi r^2$, where $r$ is the radius of the aperture. The data are well described by the power law

$$M(r) \propto r^{d_f} \propto A^{d_f/2},$$

with $d_f = 1.68 \pm 0.04$. An average for four cells yields $d_f = 1.7 \pm 0.1$ (standard deviation).

The fractal dimension for *Aplysia* neurons cultured from adult animals was, within error, identical to that reported[1,4] for adult retinal ganglion neurons *in vivo*. Further, the dimension for the cultured *Aplysia* cells was different from that reported[1] for cultured embryonic ganglion cells. These results suggest that differences in the outgrowth from adult versus juvenile neurons is not an artifact of tissue culture. Thus the difference in dimension found by Caserta *et al.*[1] for cells *in vitro* versus *in vivo* is likely to reflect differences in developmental capabilities of embryonic versus adult mammalian neurons. Further support for this conclusion comes from the results of Montague and Freedlander.[4] It is interesting that the fractal dimension observed for adult *Aplysia* neurons is equal to the dimension found for models of DLA.

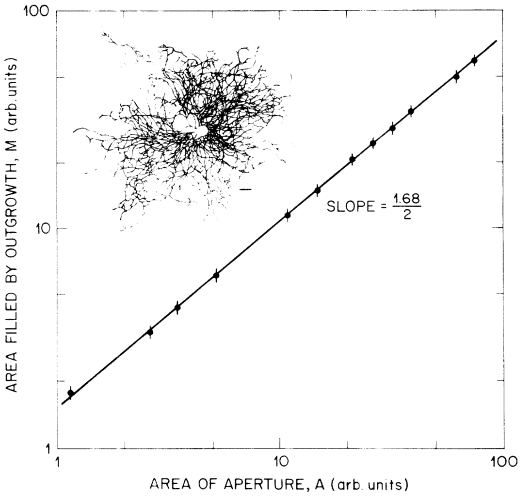


FIG. 1. The area filled by the outgrowth of a neuron (inset, bar = 100 $\mu$m) as a function of the area of apertures concentric with the cell body. The error bars represent the uncertainty in the counting process. The finite extent of the outgrowth caused the value of the filled area $M$ to deviate toward an asymptotic limit for $A > 100$; these values were excluded from the plot.

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[1] F. Caserta, H. E. Stanley, W. D. Eldred, G. Daccord, R. E. Hausman, and J. Nittmann, Phys. Rev. Lett. **64**, 95 (1990).

[2] T. Vicsek, *Fractal Growth Phenomena* (World Scientific, Teaneck, NJ, 1989).

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

[4] P. R. Montague and M. J. Freedlander, Proc. Natl. Acad. Sci. U.S.A. **86**, 7223–7227 (1989).

[5] A. Kay and R. S. Wong, J. Neurosci. Methods **16**, 227–238 (1986).

[6] D. F. Ready and J. G. Nicholls, Nature (London) **182**, 67–69 (1979); S. Schacher and E. Proshansky, J. Neurosci. **3**, 759–763 (1983).

[7] S. Schacher, J. Neurosci. **5**, 2028–2034 (1985); D. Kleinfeld, F. Raccuia-Behling, and H. J. Chiel, Biophys. J. **57**, 697–715 (1990).