Title: Fractal analysis: A useful tool for neuropathology diagnostics
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Fractal Analysis: A useful tool for neuropathology diagnostics

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Abstract

A variety of pathological changes have been described in the brains of chronic alcoholic patients. Image processing using the fractal dimension (D) as a feature parameter provides a robust method to characterise and compare the dendritic arborisation of cells of the superior frontal gyrus (Brodmann Area 8) and motor cortex (Brodmann Area 4) from controls and patients with alcoholism. Cells from BA8 in the control group were significantly different (D = 1.29) compared to cells from BA4 (D = 1.25) (n = 56; p = 0.003). D from alcoholic tissue in BA8 was significantly lower compared to control (1.25 and 1.29; n = 51; p = 0.001). This was noted for BA4 as well (1.23 and 1.25; n = 54 p = 0.02). Fractal analysis distinguished between cells in BA8 and BA4 and identified a difference in susceptibility to alcohol between the cells in these areas, BA8 showing a greater difference between control and cells of alcoholics for D compared to BA4.

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1 Introduction

Traditionally, functional differences reported for neurones in different cortical areas were attributed to the sources of their inputs and cortical circuitry and dendritic organization of the neurons were considered to be uniform.[1,2] More recently, a systematic investigation of individual neuron features in different cortical areas and species has revealed significant differences.[3] These differences are thought to be important in determining the functional abilities of neurones, cortical circuitry within areas, and processing across multiple areas.[4,5] Mechanisms that result in these areal/regional differences in pyramidal cell structure and effects of pathophysology have been largely unexplored. Various studies have reported that the number of dendrites within the arbour of cortical layer III pyramidal cells increases through development to a peak before declining throughout old age.[6] However, with the exception of a few notable studies[4,7], this research has been restricted to a single cortical area, usually a primary sensory area. [8-10] Consequently it is currently not known whether the differences in mature pyramidal cell structure result from scaling or represent fundamentally different branching patterns.

The fractal dimension (*D*) reveals something about an object not otherwise apparent. This, combined with the seemingly simple procedures involved in fractal analysis, has led to the popularity of this procedure for pattern analysis and classification tasks.

Dendritic morphology plays an important part in information processing of the neuron and classification based on the topological characteristics of the dendritic arbor adds to our understanding of neural structure-function relationships.[11,12] Recent developments in computer assisted morphological analysis has extended the number of measures available to define dendritic branching patterns, enabling the distinction between scaled and non-scaled morphologies.[13-16] Using one such feature, the fractal dimension (*D*), preliminary results of fractal analysis suggested that regional and species specialization in the pyramidal cell phenotype observed in monkeys are not attributed to scaling but reflect fundamentally different structures.[17] Here we extend this line of investigation to human cortex by using fractal analysis to determine possible scale invariance in the branching structure of layer III pyramidal cells in BA4 and BA8 of human cortex. Thus studies of single neurons of controls and alcohol affected brains may constitute a good model to study whether neurons are more prone to undergo restructuring or rescaling in association with alcoholism.

2 Method

Preserved tissue (formalin fixed) obtained from normal adult brains was provided by the Department of Pathology at the University of Sydney. All tissue was obtained in accordance with university ethics requirements.

Tissue was taken from the superior frontal gyrus (Brodmann's area 8) and primary motor cortex (Brodmann's area 4) of the left hemisphere of 14 cases (males) that were matched for post mortem delay (mean ± s.d.: 26 ± 9 hrs). The blocks were then sectioned at 100 micrometres and processed by the modified Golgi technique.[11] The modified Golgi method stains neurons, which were only included for study if they were of the pyramidal type, the cell body was located in cortical layer III, and the dendritic arbour was fully impregnated. Cells were drawn (x1000 power) with the aid of a camera lucida. Images were digitised using a flat bed scanner (72 dpi) and saved as TIF files (Adobe Photoshop software, version 6, City, USA). The cells were then skeletonised with NIH Image software (http://rsb.info.nih.gov/ij/), reducing dendritic width to a single pixel. The skeletonised images were then processed by the dilation method. The dilation method replaces each pixel of the skeletonised dendrites by circles of increasing diameter (3 to 61 pixels). The gradient (m) is computed from the regression line obtained by plotting the logarithmic values of size of the total dendritic arbour versus the logarithmic values size of the progressively larger circles. The D value of each cell was calculated as $2 - m$. Statistical differences were analysed using the S-Plus software package (SPSS Inc., Version 10, USA). A student t test was used to assess whether cell groups belonging to different cortical areas showed a significant difference in their values.

3 Results

A total of 54 cells were analysed. Statistical analysis using a Student's T test with unequal variance revealed a significant difference between the two populations of cells. From table 1 it can be seen that the D values of cells in BA4 were less than those in BA8 in control tissue. For the alcohol tissue D for the BA8 cells remained higher compared to the BA4 cells. However the change in D was greater for the BA8 cells indicating that these cells were more affected by alcohol compared to BA4 cells.

Table 1: Results for fractal analysis using dilation method.

BA*	Control	Alcohol	p
BA8	1.29±0.05	1.25±0.05	0.001
BA4	1.25±0.04	1.23±04	0.02
p**	0.003	0.29	

*Brodmann's Area; **significance value

Differences in the branching structure that equate to these differences in D value are illustrated in figure 1, where a single cell from both areas with a D value approaching the mean D for each area is illustrated.

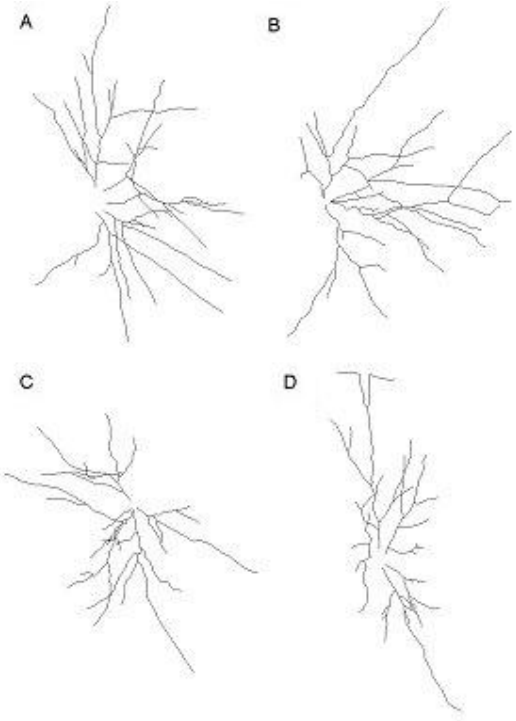

Figure 1: Skeletonised cell representatives with cell body and axon removed. BA8 control and alcohol (A & B); BA4 control and alcohol (C & D). Cell images indicate complexity of dendritic branching and not reproduced to scale.

The corresponding log-log plots obtained for the above cells are shown in Figure 2 with the regression line being indicated by the standard formula $y = mx + b$.

4 Discussion

The present results confirm and extend previous findings of regional specialization in pyramidal cell structure in human cortex.[4]

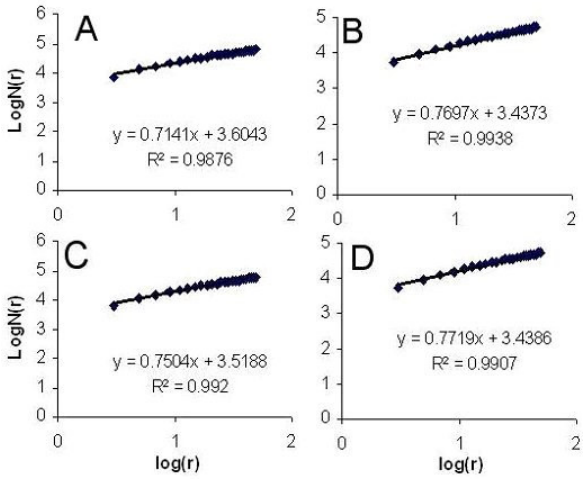

Figure 2: Log-log plots of control and alcohol layer III pyramidal cells from dilation method. A & B: BA8 control and alcohol sample cells; C & D: BA4 control and alcohol sample cells.

More importantly, our results, using a different morphological descriptor (*D*) provided evidence that regional specializations in pyramidal cell structure does not result from scaling in cell size.[19] Taken together, these data suggest that differences in the pyramidal cell phenotype in different cortical areas cannot be explained solely by the size of the brain, or even relative expansion of a particular cortical region. Instead, pyramidal cell structure is, in many cases, structurally distinguishable in different cortical areas and species due to functional requirements and interaction with adjoining cells.

Differences in the branching structure of pyramidal cell in the different cortical areas may influence neuronal function in various ways. For example, differences in the number of branches within the arbour result in differing ability to compartmentalize the processing of inputs, endowing more branched cells with greater functional capacity than less branched cells.[20] Studies of other neuronal types have shown a correlation between *D* and other aspects of cellular function. *D* has been shown to correlate with differences in Muller cell coupling in the retina, which influences the propagation of the excitation waves.[21] The length constant of cable core-conductance of currents, which plays a role in spatial buffering of potassium currents has also been correlated with *D*.[22,23] Similarly, passive properties of the dendritic tree have been associated with the *D* value of cells in the fish retina.[24] The space filling properties of dendritic arbours, as revealed by fractal analyses, may also provide a measure of circuit complexity. For example, neurones with a higher *D* value have a greater space filling capacity as compared with those with lower *D* values. As approximately 70% of neurones in the cerebral cortex are of the pyramidal type, circuits composed of individual neurones with relatively high *D* values potentially have a greater density of neurites within the cortex than circuits composed of neurones with relatively low *D* values.

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