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BOX-COUNTING ANALYSIS OF MICROGLIA FORM IN SCHIZOPHRENIA, ALZHEIMER'S DISEASE AND AFFECTIVE DISORDER

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Abstract

In pathological brain, a variety of morphological forms exist that reflect differences in functional requirements. To better understand microglia function in neurological disease, it is important to identify and quantify microglia morphology associated with specific neuropathologies. Traditional feature parameters such as area or cell diameter are not sufficient. In this study microglia were quantified by the box-counting fractal dimension (D_B). One hundred and four cells from post-mortem tissue were analyzed comprising cells of controls, Alzheimer's disease, schizophrenia and affective disorder. The D_B was significantly different from the control (1.36) compared to schizophrenia (1.41), Alzheimer's disease (1.41) and affective disorder (1.43) with $p < 0.01$. Thus fractal analysis provides a useful quantitative and objective measure of microglial form associated with normal function and diverse neuropathology. The distribution of fractal dimensions associated with microglia structure and activation with disease progression also differs, suggesting a different etiology for these diseases.

Keywords: Microglia; Morphology; Fractal Analysis; Neuropathology; Schizophrenia; Alzheimer's Disease; Human.

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1. INTRODUCTION

Microglia are small central nervous system (CNS) immune effector cells. They can maintain, disrupt, or destroy CNS function.[1] Deciding whether microglia are active or distinctly different in form as part of a specific pathophysiological process or different with respect to disease entity is most often determined using biochemical markers.[2] The decision can become rather complicated due to the biochemical repertoire and the morphology of microglia depending on factors such as pathological stimuli, age, diet, brain location and gender.[3] In addition, differentiating small differences in microglia structure has not been possible using conventional morphological parameters such as area or density measures.

Microglial morphology is traditionally classified as ramified, bushy, or reactive, corresponding to a spectrum within which cells range from passively monitoring to actively modifying brain structure and function (Fig. 1).

As evident in the general trends shown in Fig. 1, in normal adult human brain and spinal cord, small-bodied cells with long, finely branched processes are presumed to be "resting", whereas in pathological CNS, "reactive" cells, which have begun to swell and wind in their processes, or may have completely lost them, are assumed to be "active".[4] However

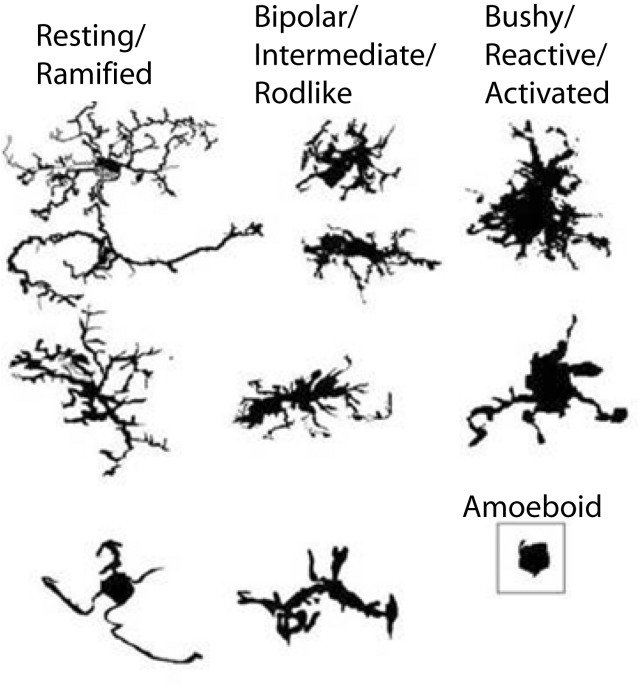

Fig. 1 Morphological characteristics of microglia.

microglia may be in a number of different stages between and including the resting and active state at any one time. At the margin where resting merges with activated, branches are shorter and thicker, somata are bigger than in normal microglia, but cells are not clearly "activated". Microglia in this state may be responding mildly or returning to resting,[3,5] but in schizophrenia and perhaps other cases such as Alzheimer's disease, microglia may be behaving quite differently.[3,5,6]

In schizophrenia, some studies have suggested no obvious pathological changes associated with microglia nor an increase in microglia detected by certain staining methods in certain brain areas. This was interpreted as suggesting that microglia are not abnormally activated in at least some forms of schizophrenia.[7,8] Similar findings have been reported for Alzheimer's disease.[9,10]

It is important, then, to be able to assess even subtle changes in microglial morphology and relate these to potentially pathological events. Fractal analysis assesses the relative complexity of form, such as the border invaginations of a cell, quantitating features that are often difficult for observers to describe. The fractal dimension has been used to differentiate subtle morphological differences and function in glia and neurons.[11–14] A study of Müller cells in chicken retina has indicated that the magnitude of the fractal dimension correlates with differences in coupling between cells in the periphery versus central retinal regions.[15] Changes of cytoplasm and surface antigen expression in microglia may also be correlated to membrane conductance, which in turn is correlated with the fractal dimension.[16,17] In further studies, a link between the fractal dimension and the surface-to-volume ratio, which in part determines the conductance along a membrane and plays a role in spatial buffering of K^+ currents, has been shown.[16,18,19]

Recently, Soltys *et al.* showed that the fractal dimension detected subtleties people overlooked between ramified microglia from compromised and uncompromised animals.[13] We expanded this work by illustrating that the range of microglia morphologies associated with different human neuropathologies have unique signatures. The research reported here investigated how well fractal analysis detects subtle differences in microglial structure in three pathological paradigms and indicated that different neuropathological states are associated with different morphological characteristics based on the fractal dimension.

2. MATERIALS AND METHODS

We analyzed 104 published images of cells from post-mortem tissue of control ($N = 21$) as well as pathological tissue (schizophrenia $N = 43$; Alzheimer's disease $N = 19$; and affective disorder $N = 19$).[20,21] This provided a good range of cell morphologies from normal and pathological brain tissue. Cell images were scanned into an IBM P4 computer using Adobe Photoshop LE (V5.0). Information for the fractal analysis was extracted from digital images using the following method based on one described previously.[22] Each cell, using Adobe PhotoShop LE (V5.0) and a Wacom computer graphics tablet, was converted to a grey-scale image, the background subtracted using automatic filtering, and the resulting image converted to a binary contour. The box-counting dimension (D_B) for control and pathological cells was obtained using the public domain program, ImageJ, and the plug-in, FracLac,[23] written in our laboratory for analyzing morphological complexity of biological cells (both programs are freely available online from the US National Institute's of Health, at http://rsb.info.nih.gov/ij and http://rsb.info.nih.gov/ij/plugins/fraclac/fraclac.html).[12,19,22]

In box counting, data are gathered by laying boxes over a digital image as a series of grids of decreasing box size, then the number of boxes that fall on the image (N_C) and the size of each box (C) are recorded. C, the relative scale, can be considered as 1/box size, because the image size is a constant. From this series of paired data, one infers the D_B as the slope of the log-log plot of C^{-1} on the x-axis and N_C on the y-axis.

3. RESULTS

Figure 2 shows the continuum of D_B values obtained for control cells in our study.

Differences were significant for disease groups compared to control states (Fig. 3).

Analysis of variance showed that complexity as measured by the box-counting dimension was significantly different from control for all disease states (schizophrenia, Alzheimer's disease and affective disorder) at $p < 0.01$. Table 1 shows these results.

4. DISCUSSION

Differences in microglial morphology are visually distinguishable in grossly pathological tissue, as in Alzheimer's disease, but are less obvious, and more debated, in other types of pathology, such as schizophrenia and affective disorder. It has increasingly become evident that microglia can be active without proliferating, and that not all visualization methods reveal microglia equally at all stages of activation or in all brain areas.[3,24] Moreover, convincing recent evidence suggests at least some microglia in some cases of schizophrenia are unusual, where, in particular, microglial morphology may be subtly altered.[21]

Using box counting to determine the fractal dimension of human microglia, fractal analysis differentiated microglia in both grossly pathological and subtly affected brain from microglia in control brain, even when differences were not clearly evident on inspection (see Table 1). As can be discerned from the peaks of the individual traces in Fig. 3, the D_B index differentiated the overall

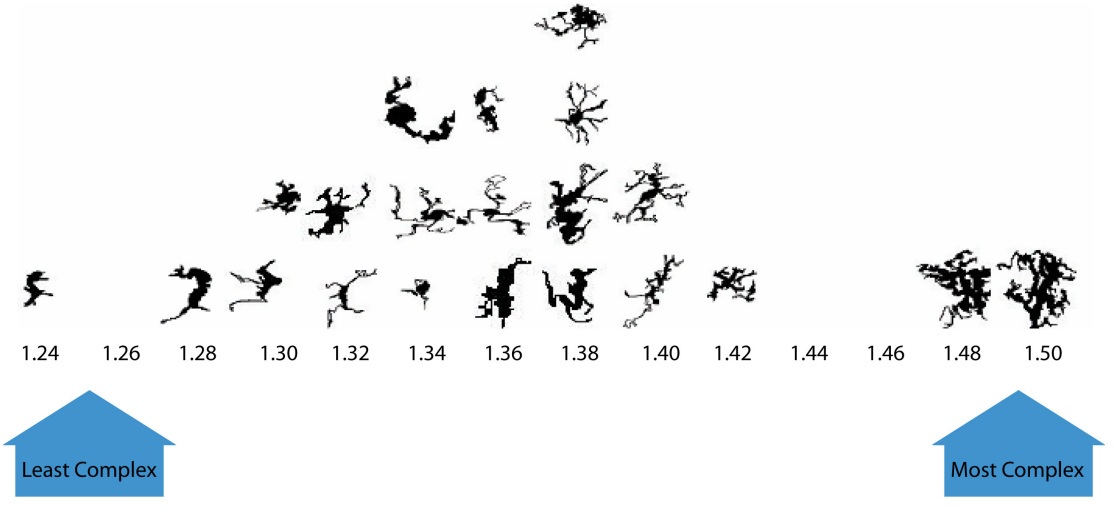

Fig. 2 The box-counting fractal dimension ranks complexity of microglia in control human cortex.

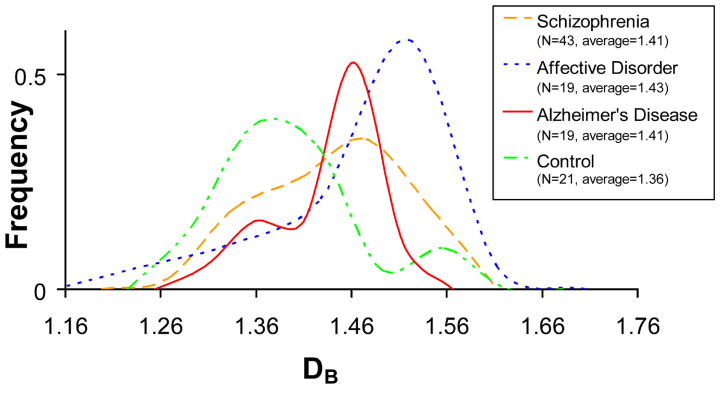

Fig. 3 Changes in D_B associated with disease.

Table 1 Results of Fractal Analysis for Neuropathology Groups.

	Control	Schizophrenia	Alzheimer's Disease	Affective Disorder
Mean D_B^*	1.36	1.41	1.41	1.43
Median D_B	1.35	1.42	1.42	1.46
Standard Deviation	0.07	0.07	0.05	0.09
Range	0.26	0.28	0.2	0.37
Minimum	1.25	1.25	1.3	1.17
Maximum	1.51	1.52	1.5	1.54
Count	21	43	19	19
CI (95.0%)	0.03	0.02	0.02	0.04

*Box-counting fractal dimension.

distributions of complexity in microglia in human brain under a variety of pathological conditions, and quantitated differences between subtly activated and normal cells. While our data suggest that complexity is a useful guide for objectively quantitating normal and abnormal microglial morphology, it remains to be determined what mechanisms and meanings underlie subtle morphological changes in microglia associated with pathology.

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