Mathematical modeling and computational analysis of neuronal cell images - application to dendritic arborization of Golgi impregnated neurons in dorsal horns of the rat spinal cord

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Received: October 12, 2004 | Received in revised form: March 23, 2005 | Accepted: April 11, 2005 [Available online: September 6, 2005]

Abstract: Neurons of the rat spinal cord have been stained using the Golgi impregnation method. Successfully impregnated neurons from laminae I to VI were subjected to a computational analysis for complexity of dendritic tree structure. The analysis was performed using ruler-counting and circle-counting techniques. Our analysis aimed to support quantitatively the general concept of Rexed’s laminar scheme of the dorsal horn of mammals. For that purpose, we have developed two mathematical models of neuronal arborization patterns, whose solutions yielded the inverse powerlaw and generalized power-law scaling. The latter comprises two main parameters: (i) the anfractuosity (A), characterizing the degree of dendritic complexity and (ii) an estimate of the total length of arborized dendrites. The anfractuosity can distinguish among the sets of drawings over all six laminae. – Neurocomputing 69: 403–423 (2006) [doi:10.1016/j.neucom.2005.04.007]

Keywords: Dendritic arborization; Golgi method; Mathematical modeling; Rat spinal cord; Rexed’s scheme

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One of the major goals in cellular neurobiology is meaningful quantitative analysis of neuronal arborization patterns. Sholl’s [41] consecutive-circle (cumulative intersection) method specifies dendrite geometry, ramification richness, and branching patterns. This analysis, both manual [40, 41] and computer-assisted [6, 15, 31, 35], has long been widely applied and used for quantitative morphometric studies of Golgi-impregnated cells [8]. Mandelbrot’s [33] fractal theory analyses the neuronal arbors for fractality [8, 9, 35, 43, 49], and classifies neurons according to their fractal dimensions [8, 16, 25, 26, 35, 50]. In order to analyze the overall shape complexity of neuronal cells, the wavelets and multi-scale curvature methods were used [9]. The characterization of neurons by means of semi-automated generation of dendrograms, describing the branched structures of neurons in terms of the lengths, average thickness, and “branching energy” of each of the dendrite segments, is also described [10]. This “energy” quantifies the complexity of the shape and can be used to characterize the spatial coverage of the arborization. The spectral method for estimating geometric structures of contours and the use of exact dilations to estimate geometric measures were recently offered [11]. For the quantification of neuronal shapes, the segmentation of neuronal objects from confocal digitized images is also performed [49]. Various computer software for digitization and neuron analysis have been designed. Special attention was paid to the dendrite reconstruction and its visualization on screen mainly after applying the method of Golgi impregnation. Outputs are given either to a color plotter or to laser printers in graphical form as a summary of various metrical and topological parameters [24, 31]. However, most of the authors use direct-measurement techniques. Usually, the quantitative descriptions of neuronal arborization, as offered by many authors, are almost entirely based on the analysis of somata sizes, their location and grouping, dendrite arborization sizes and bifurcation ratios, total dendrite areas, number of branching points, etc [17, 21, 24, 32, 41].

Mathematical modeling, as a quantitative method, is also applied to neurobiological studies. For example, the mathematical modeling approach to determination of how the morphology of the dendrite population affects the velocity of RNA transport in neurons is successfully used [14]. The geometrical branching pattern of the dendrites is also analyzed adopting a mathematical model that incorporates random and deterministic effects [48]. The mathematical QS-model of dendrite growth as a sequence of branching events at randomly selected segments is presented. It is shown that the variation of the number of dendrite segments can be accounted for by assuming that new branches during neuronal outgrowth tend to be formed randomly at terminal segments [36, 37]. Besides that, growth processes can be successfully modeled as Lindenmayer systems [23]. MicroMod is web-based software that allows models to be easily built using a comprehensive set of adjustable parameters [23]. Jelinek et al [23] suggest some of the numerous applications for this approach to neuronal modeling. A suitable polynomial function is used as a fitting mathematical model to estimate some morphologic parameters characterizing dendritic arbor complexity [39]. Mathematical and computational modeling, including L-systems, has recently emerged as a powerful approach to the quantitative anatomical characterization of dendrite morphology [3]. Burke and Marks [7] investigate the relationship between dendritic structure and its morphologic patterns, and neuronal packing in spinal motoneurons. Analysis of the effects of dendrite complexity on network connectivity is oriented toward the issues of neuronal shape characterization and cell geometry at the individual cell level. This analysis is carried out by means of critical percolation probability [12]. Other models concerning neuronal arborization have also been developed [29, 30]. In qualitative analysis of dendritic arborization patterns, neurons are mostly studied in relation to their localization in the nervous system [52], particularly in certain regions and laminae [40, 52]. It seems that each lamina possesses a comparatively distinct dendrite organization. The
laminar structure of the spinal cord is related to the morphological classification of spinal neurons and dendrite tree geometry. Recent studies mostly analyze the morphology of neurons in lamina I [13, 18, 52]. As for the dendritic arborization of cells in the lumbar spinal cord of the rat, little analytical evidence is available on neurons in relation to their laminar organization. In most studies, the morphology of cells in the rat dorsal horn is considered and analyzed only over lamina I [27, 28, 34, 51]. Neurons extending over laminae II and III (substantia gelatinosa) are also described [5]. To our knowledge, there is little published information on the applications of quantitative methods to the laminar classification of neurons and dendrite arborization patterns in the rat.

In the present study, we develop two mathematical models whose solutions are used for the morphometric computational analysis of dendritic organization of neuronal arbors in dorsal horns of the rat spinal cord. Our analysis aims primarily to support quantitatively the concept of Rexed’s lamination of the dorsal horns of mammals. Through the analysis of such laminar organization, we show that the proposed method is sensitive in practice, enabling a rather precise discrimination of neurons over the laminae of the dorsal horn.

**Histology**

Tissue samples of lumbar spinal cord of adult Wister rats weighting 200–250 g were used in the present study. Formaldehyde tissue fixation and Golgi staining techniques were performed as previously described [39, 45]. A total of 15 spinal cords with satisfactory impregnations of dendritic trees were used. Excised spinal blocks, 3–5 mm long, were further processed. Sectioning was enabled by immersing whole blocks in liquid paraffin at 56 C. Prepared tissue blocks were fixed on a microtome and sectioning was done at 90–120 /um thickness. The sections were placed on glass slides, covered in DPX resin (Fluka) and analyzed using the light microscope. The cord was studied mainly in sagital sections. Twenty-three representative and successfully impregnated neurons were selected and traced under a magnification of 500 x with the aid of a camera lucida attachment (Zeiss drawing tube), at an objective magnification of 40 x. The magnification scale was indicated by bars corresponding to 10 and 25 /um lengths. Incomplete impregnation can influence the quality of measurements. To overcome this, special care was taken in the choice of representative neurons — such where by our judgment these problems were minimal.

In accordance with other studies, impregnated dorsal neurons have shown mainly fusiform, stellate – fusiform, and stellate-pyramidal constellations of cell bodies and primary dendrites. The present study is concerned with the morphology of laminae I–VI of the dorsal horns. The neurons were classified according to the Rexed laminar concept. For that purpose, all neurons were pooled in five groups according to their laminar position: (i) four neurons from spinal lamina I (the marginal layer), (ii) seven neurons occupying spinal laminae II and III (the substantia gelatinosa), (iii) five neurons from lamina IV, (iv) three neurons from lamina V, and (v) four neurons from lamina VI. This set of neurons was taken from our previously published article [39] and other official sources [19, 20]. The multi-polar organization of lamina IV cells contrasts with the gelatinous elements above (laminae I–III), representing sensory (afferent) neurons, and with the interneuron of deeper laminae (laminae V and VI), conducting impulses from sensory to motor (efferent) neurons. The neurons over lamina IV represent a mix of the two.
**Fig. 1:** Derivation of the model of scale-counting. The number of ruler (scale) steps $N$ is plotted against the ruler length $r$ (A), and the ratio $\frac{\Delta N}{N}$ is plotted against the ratio $\frac{\Delta r}{r}$ on Cartesian axes (B). The plot (A) is obtained by fitting (3) to data points (open circles), and the plot (B) is drawn using (1) (filled circles). $R$ is the coefficient of correlation. The proportionality factor between real dendrite size and traced drawing: 25 $\mu$m $= 19$ mm.
Fig. 2: Number of ruler steps $N$ (left axes, full lines) and length of dendrites $L$ (right axes, dashed lines) of two neurons, plotted against the ruler length $r$ on log–log axes. Full lines are obtained by fitting (4) and dashed lines by fitting (7) to data points. $F$ is constant ratio for an inserted neuron. Proportionality factor between real dendrite size and traced drawings: 25 /um = 45 mm (A) and 25 /um = 46 mm (B).
Fig. 3: Derivation of the model of scaling. The length of dendrites $L$ of a neuron is plotted against the ruler length $r$ (A), and the ratio $DL/(CL)$ is plotted against the ratio $Dr/r$ (B). The first plot (A) is obtained by fitting (7) to data points (open circles), and the second plot (B) is drawn using (5) (open circles). Proportionality factor between real dendrite size and both traced drawings: $25 \, \mu\text{m} = 19 \, \text{mm}$. 
Fig. 4: Preparation of camera lucida drawings. A drawing is converted into digital image (A) and imported into the ImageJ program. Axons and soma are digitally removed and each dendrite is filled with pixels (B). The program reduces the image to a skeleton (C). For dendrite branches (if exist), pixels connecting the branches to the dendrite stems are removed. The segments 3, 4, and 7 are broken off from their main stems 2, 2, and 6, respectively, by removing pixels denoted by arrows (D). If two dendrites intersect, two pixels from one of them, joining the other, are removed (E, dendrite 5). The removed pixels are shown by two arrows (E).
Fig. 5: Comparison of the three methods used in neuronal images processing. Total length $L$ of the dendrites of a neuron is plotted against the ruler length $r$. All the plots are obtained using (7). The result of measuring the real drawing using the divider is shown by a dotted plot (squares), that of skeleton image by a dashed line (filled circles), and that of the circle-counting method, by a full line (open circles). Arrows show the position of intersection of the plots with the vertical axis. The subscript IJ denotes ImageJ. The values of anfractuosity $A$ and dendrite length $C$ estimated from (7) are presented in the inset.
Fig. 6: Silhouettes of drawings of Golgi-impregnated lamina I (A) and laminae II–III (B) neurons from dorsal horns of the rat spinal cord. Vertical axes (left and right) show the anfractuosity $A$ and vertical axis in the middle shows the ratio $F$. Scale bars: 25 $\mu$m for neurons 1 and 3, 10 $\mu$m for neurons 2 and 4 (A), and 10 $\mu$m for all neurons (B).
Fig. 7: Silhouettes of drawings of Golgi-impregnated laminae IV (A), V (B), and VI (C) neurons from dorsal horns of the rat spinal cord. Vertical axes (left and right) show the anfractuosity A and the two vertical axes (middle) show the ratio F. Scale bars: 25 μm for neuron 1, 10 μm for neurons 2-5 (A), and 25 μm for all neurons (B and C).
Discussion

We have pointed out that the anfractuosity (A) of a neuronal border was proven to be a useful descriptor of the cell’s complexity. This raises the question of what aspect of the cell’s “complexity” is the anfractuosity measuring: what does “complexity” actually mean, apart from being a notion opposite to “simple”? There have been attempts to define this notion exactly [44, 47]. In the present study, the anfractuosity (A) has proven to be a useful tool in quantifying the degree of global or average dendrite aberrance from straight lines. Although mechanisms and basic nature of neuronal Golgi impregnation have been established only recently [45], it is generally accepted that Golgi impregnation satisfactory reveals naturally occurring neuronal structures and dendrites branching. One of the predictions of this finding is that the Golgi impregnation method permits an acceptance of the anfractuosity (A) in neuronal studies. Besides, outcomes from our preceding studies [39] enable an adoption of the anfractuosity as one of the basic parameters in neurocomputational investigations.

To test the properties of dendrite drawings with mathematical model (7) for differences among five groups of neurons, we have used, in essence, one of the fractal length-related techniques known as the trace (ruler-counting) method [4, 8, 16, 22, 39, 44]. This authentic method is based on the use of a divider for dendrite lengths measuring. It has been noted that the major drawback of this method was inaccuracy in processing the neuronal images; some other length-related techniques seem to be superior [16, 43, 44]. To measure the dendrite lengths by means of a divider, we have used a rather broad interval (from 2 to 25 mm) for the divider spans. Although only a part of this data is presented in Fig. 5, all of it is used for fitting model (7). If we mathematically extrapolate this graph to the section with the ordinate axis, unreal (extremely large) values for length estimate C are obtained (see the inset in Fig. 5). This is another shortcoming of the ruler-counting method. Besides, despite the use of such a long interval of divider spans, the starting dendrite length L (of 345 mm, see the graph in Fig. 5) is abbreviated only for 10%. In this respect, we can consider that this length is approximately constant. Under this assumption, it follows from model (7) that the length estimate C is inversely proportional to the anfractuosity A. Indeed, if the values of A are equal to unity, the graph of the model is a decreasing straight line. For A>1, the graph is a convex-upward parabola with the smaller value of C. But, if these values of A are less than 1 (being just our case), the graphs are concave-upward curves, so that the values of C must be larger than previously. Therefore, the small (unreal) value for A obtained when the ruler-counting method is used seems to be the consequence of the (also unreal) value for C estimated using this manual technique for length measuring. Use of the software for circles drawing eliminates all these shortcomings.

The laminar organization of the spinal grey matter, described in the cat by Rexed [38], has been rapidly adopted by most neuroanatomists because it avoids the problems of varied nomenclatures of classical anatomic descriptors. But, one could question whether data obtained in the cat can be generalized to other animals, because it has been noticed that Rexed’s laminar scheme can be found in all mammals but not in other vertebrates [40]. Our quantitative morphologic analysis of the rat spinal cord favors the Rexed [38] concept on laminar cytoarchitectonic scheme, since each lamina I–VI of the rat testifies to a quite specific dendritic organization. This is in full agreement with the qualitative observations made in the cat [38, 46] and human [40]. We cannot directly compare our results for complexity of dendrite arbors with those of others, but all main types of substantia gelatinosa neurons (“islet” and “type III stalk” cells) have short longitudinal dendrite arbors [5]. This result is in accordance with our finding that the total lengths of arbor dendrites extending over the substantia gelatinosa are the smallest as compared to these of neurons from other laminae (Table 3). Although the number of neurons used in
this study could be considered as comparatively small, with our statistical analysis of groups of cells of different morphologic types, the significance of differences in the anfractuosity $A$ for different types of cells is exactly determined. The methods of statistical analysis used here have demonstrated that the six laminae we have analyzed differ in their anfractuosity. These values of the $A$ support the concept that these cell groups represent populations of different neurons that can be clearly discriminated from one another. Our conclusions concerning the analysis of dendritic complexity of neurons over these six laminae are given within precisely determined statistical limits, with levels of significance of $p^* < 0.05$ and $p^{**} < 0.01$. This conclusion emphasizes our impression that further effort in neuroscience should be directed toward the continuation of these investigations.

References

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