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BRANCHING-PATTERN ANALYSIS OF THE DENDRITIC ARBORIZATION IN THE THALAMIC NUCLEI OF THE RAT BRAIN*

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We investigated the dendritic patterns of rapid Golgi-impregnated, highly similar multipolar neurons from two functionally different thalamic regions of the rat brain: two dorsal nuclei (the nucleus laterodorsalis thalami, pars dorsomedialis and the nucleus laterodorsalis thalami, pars ventrolateralis), and two ventral nuclei (the nucleus ventrolateralis thalami and the nucleus ventromedialis thalami). The analysis involved conventional morphometric parameters (height and size) and a new parameter derived from graph theory, the relative imbalance (RI), derived from the branching patterns of the dendrites, which permits quantitative characterization of the dendritic arborization of a neuron. On this basis, neurons can be grouped into three fundamentally different types: type A, or highly-polarized (imbalanced) neurons (RI values close to 1); type B, or medium-polarized neurons (RI values around 0.5); and type C, or balanced neurons with low polarization (RI values close to 0). The orientations of the dendritic arbor, and thus the receptive fields, of the dorsal and ventral thalamic neurons, were mutually perpendicular. The H and S values indicated that the neurons in the dorsal and ventral thalamic nuclei differed significantly. However, their RI values demonstrated that they were similar neurons of type B. Our data reveal that 1) the dendritic arbor cannot be reliably characterized purely on the basis of height and size, and 2) RI is a valuable morphometric parameter that identifies the true nature of the dendritic arborization.

Keywords: Dendritic arborization – Golgi impregnation – morphometry – rat – relative imbalance – thalamus

INTRODUCTION

Numerous quantitative studies have been performed on the morphometric parameters of the different cellular characteristics of the neuron (perikaryon, axon, dendritic arborization, etc.). A quantitative description of its dendritic arborization is highly relevant, since such information can be utilized for a functional characterization of the network activity, synaptic connections and associated physiological features. Such morphometric parameters include, but are not restricted to, the area, the height, the radius, the branch length, the numbers of branch points and segments, and the number of spines of the dendritic tree, and the orientation histograms and convex hull (see

*Dedicated to Professor József Hámori on the occasion of his 70th birthday.

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[5, 8, 9] and [11], and references therein). Different problems in the analysis have recently been addressed by computerized methods (for example, see [1] and [12], and references therein). However, there is no consensus as to which of the many measurable geometric parameters are the critical determinants of the dendritic arborizations. Certainly, there is no single parameter in use today that can characterize the pattern of dendritic arborization, even to a first approximation. We now introduce and test a new approach to dendritic branching analysis, involving use of the parameter relative imbalance (RI), derived from graph theory. RI alone is shown to quantitatively characterize the spatial features of the dendritic arborization of a neuron.

Our morphometric studies were focused on two functionally different regions of the rat brain where highly similar multipolar neurons reside. One of the regions encompasses two associative nuclei in the dorsal part of the thalamus, the nucleus laterodorsalis thalami, pars dorsomedialis (LDDM), and the nucleus laterodorsalis thalami, pars ventrolateralis (LDVL), which receive information from several vision-related structures, including the superior colliculus, the pretectal nuclei and the ventral lateral geniculate nucleus. The other region is the ventral part of the thalamus, containing the nucleus ventrolateralis thalami (VL) and the nucleus ventromedialis thalami (VM), which mainly relay activity from the deep cerebellar nuclei to the motor cortex and receive inputs from the globus pallidus, the endopeduncular nucleus and the reticular part of the substantia nigra (see [3] and [10], and references therein).

MATERIALS AND METHODS

The experimental procedures were carried out in strict compliance with a European Communities Council Directive (86/609/EEC), and followed the Hungarian legislation requirements (XXVIII/1998 and 243/1998) regarding the care and use of laboratory animals. Seven adult Sprague-Dawley rats (180–200 g) were used. Under slight ether anesthesia, the animals were transcardially perfused with 150 ml physiological solution (0.9% NaCl, 0.01 M Na_2HPO_4, pH 7.4), followed by 300 ml rapid Golgi fixative solution (1% glutaraldehyde, 1% paraformaldehyde, 0.12 M Na_2HPO_4, pH 7.4). The brains were removed and postfixed in the above solution for 1 day, then cut into 4–5 mm pieces and incubated in 3% potassium dichromate solution (pH 6.8), with occasional mixing at room temperature (RT). After the dichromate layer had been rinsed off the surface of the brain pieces with distilled water, the tissue was impregnated in 0.75% $AgNO_3$ solution (pH 7.4) at RT for 1 day, and then cryoprotected in 0.01 M phosphate buffer (pH 7.4) containing 30% sucrose at 4 °C for 1–2 days. The tissue was embedded in Cryomatrix (Shandon Scientific Ltd., Pittsburg, PA, USA), and frozen and frontal sections were cut (80 µm) at bregma –2.80 [7] in a cryostat, mounted on glass slides, dried at RT for 1 day and covered with Entellan (Merck, Darmstadt, Germany).

Twelve neurons from the LDDM, 8 from the LDVL, 12 from the VL and 12 from the VM were examined (see Figs 4 and 5) under a Leica DM LB light microscope (Leica Mikroskopie und Systeme GmbH, Wetzlar, Germany) equipped with a cam-

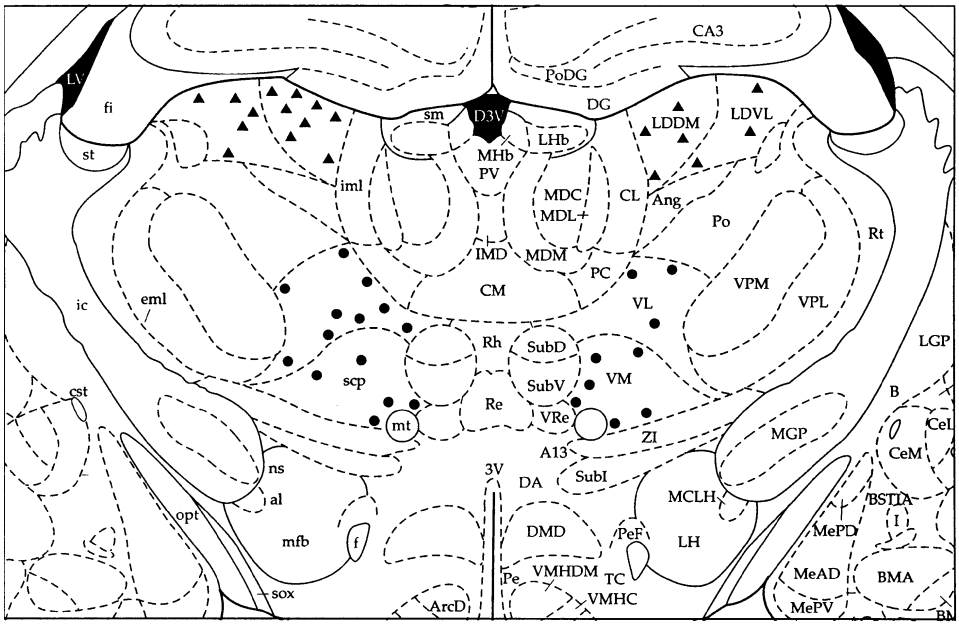

Fig. 1. Locations of the neurons analyzed in this study in the dorsal and ventral parts of the rat thalamus according to Paxinos and Watson [7]. Twelve neurons from the LDDM, 8 from the LDVL, 12 from the VM and 12 from the VL were analyzed for morphometric characteristics. ▲= dorsal neurons, ! = ventral neurons

era lucida. The locations of the neurons studied were confirmed via the atlas of Paxinos and Watson [7] and are depicted in Fig. 1. Camera lucida pictures were drawn, digitized (Microtek II HR; Microtek Int., Taiwan) and photographed by a digital microscope camera (Polaroid DMC 1; Polaroid Corp., Cambridge, MA, USA) attached to a Macintosh 8600/250 (Apple Computer, Cupertino, CA, USA).

For evaluation of the dendritic pattern by the binary tree model (see [8] for details), the following features were determined on the dendritic tree of each neuron 1) the initial point, 2) the proximal dendritic segment, 3) the dendritic node, 4) the bifurcation point, 5) the internodal segment, 6) the terminal point, and 7) the terminal segment (Fig. 2). The origin of the dendrite stem was taken as the initial point; the proximal dendritic segment lies between the initial point and the first branching point (dendritic node or bifurcation point). The terminal points are the last nodes of the binary tree, from which no further dendritic segment branches out. Thus, a dendritic tree lies between the initial and terminal points, which establishes the height (H) of the tree. The determination of H requires three segment types: the proximal segment (PS), the internodal segment (IS) and the terminal segment (TS). We selected the longest path between the initial and the farthest terminal points; each of these paths contains only one PS and TS, but the number of the internodal segments varies greatly. For the determination of H, the following equation was used: $H = n + 2$,

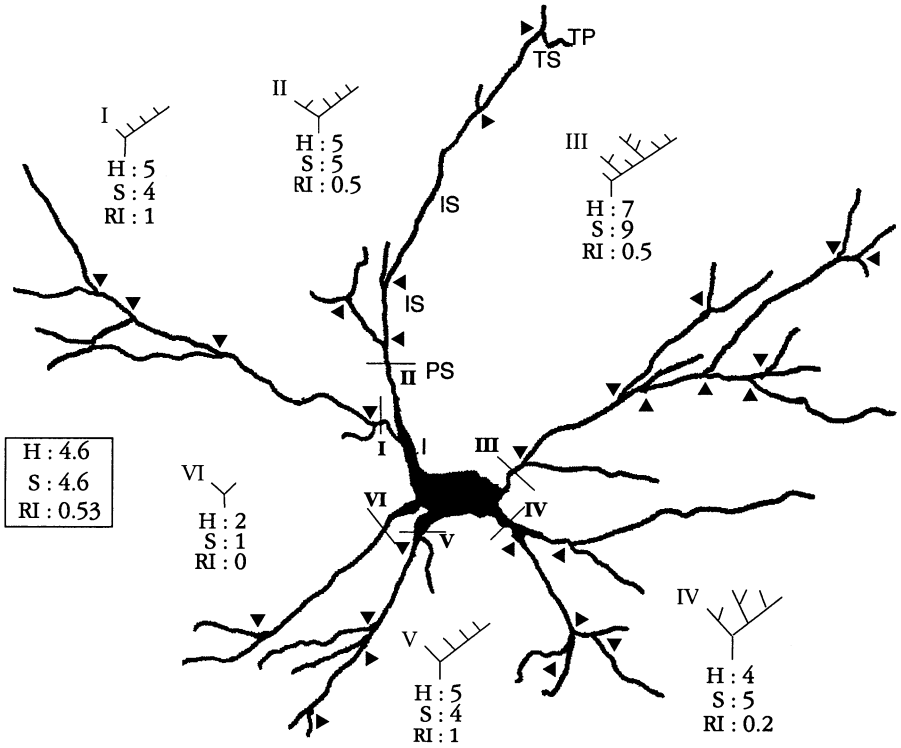

Fig. 2. Camera lucida drawing of a typical thalamic neuron (no. 1 in Fig. 5) from the VM. The individual dendritic trees (labeled by Roman numbers) are indicated together with their H, S and RI values. The values in the box are for the whole neuron. Arrowheads: bifurcation points, I = initial point, PS = proximal segment, IS = internodal segment, TS = terminal segment, TP = terminal point, H = height, S = size, RI = relative imbalance

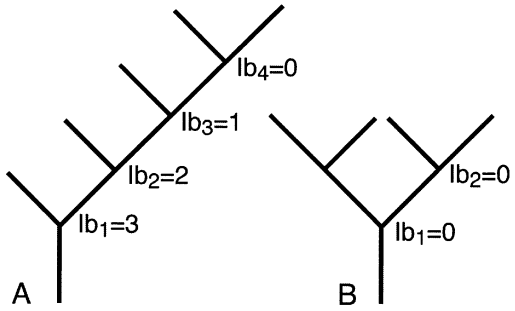

Fig. 3. Extreme values of the parameter RI in schematic dendritic trees. (A) An imbalanced dendritic tree, where the absolute value of the imbalance of the bifurcation points (Ib) = 3 + 2 + 1 + 0 = 6; the maximal imbalance of this particular dendritic tree (MId) = 6, and thus RI = 1. (B) A balanced dendritic tree, where Ib = 0, MId = 0 and RI = 0

where n is the number of internodal segments on the longest path. Three parameters were used to describe the dendritic tree in this study: H, S and RI (Fig. 2).

The RI value of a neuron was calculated as follows. First, the size (S) of the dendritic tree was determined via the total number of bifurcation points (Fig. 3). The imbalance (Ib_i) of a bifurcation point (i) was then determined by taking the absolute value of the difference of the S values of the subtrees originating from i. The imbalance of the particular dendritic tree was calculated as $Id = Ib_1 + \ldots + Ib_n$. The maximal imbalance of this particular dendritic tree (MId) was computed as $MId = S(S-1)/2$. The relative imbalance of this particular dendritic tree (RId) was calculated via the formula RId = Id/MId. The RI value of the whole neuron is the average of the RId values, $RI = (\sum (n\ Id/n\ MId))/n$, where n is the number of dendritic trees of a neuron. An example for the above calculations is shown in Fig. 3. The RI value varies between 0 and 1. If RI = 1, the dendritic tree is fully imbalanced and asymmetric, or highly-polarized, and characteristic of type A neurons (Fig. 3A). If RI = 0, the tree is balanced and symmetric or low-polarized, and is characteristic of type C neurons (Fig. 3B). A dendritic tree with an RI value of around 0.5 can be described as medium-polarized, and it is characteristic of type B neurons.

Statistical calculations were made with Excel 5.0a (Microsoft Corp., Redmond, WA, USA). Analysis of significance was carried out with the two-tailed Student's t-test at a significance level of 0.05.

RESULTS

The morphology of the multipolar neurons in the dorsal and ventral parts of the thalamus investigated in this study is depicted in Figs 4 and 5, respectively. Apart from the highly similar appearance of these neurons in both regions, their arborizations in the nuclei of the dorsal part occupied a vertical position, while those in the ventral part of the thalamus were mostly arranged horizontally. The LDDM and LDVL neurons had PS values between 3 and 8, while those in the ventral nuclei had a somewhat wider range of PS values (3–13; Fig. 6). The values of the parameters (H, S and RI) calculated to characterize the dendritic arborization are presented in Table 1. In spite of the similar general appearance of the neurons, two parameters classically used for such a characterization (H and S) indicated differences between the groups of neurons in the dorsal and ventral parts of the thalamus. The neurons from the dorsal part differed significantly in H ($p < 0.01$) and S ($p < 0.05$) from those in the ventral parts. For example, while H for the dorsal neurons was 4.75 ± 0.79 (mean ±S.D.), the corresponding value for the ventral neurons was 4.01 ± 0.87. Moreover, the difference in S indicated that the neurons from the dorsal part had more bifurcation points than those from the ventral part (5.27 ± 1.60 and 4.06 ± 1.64, respectively). However, the RI values for the neurons from these areas did not reveal any significant difference ($p = 0.22$), indicating that these neurons are of basically the same type B, with symmetric and medium-balanced dendritic arborizations characterized by average polarization and spatial distribution. The average RI values for the neurons

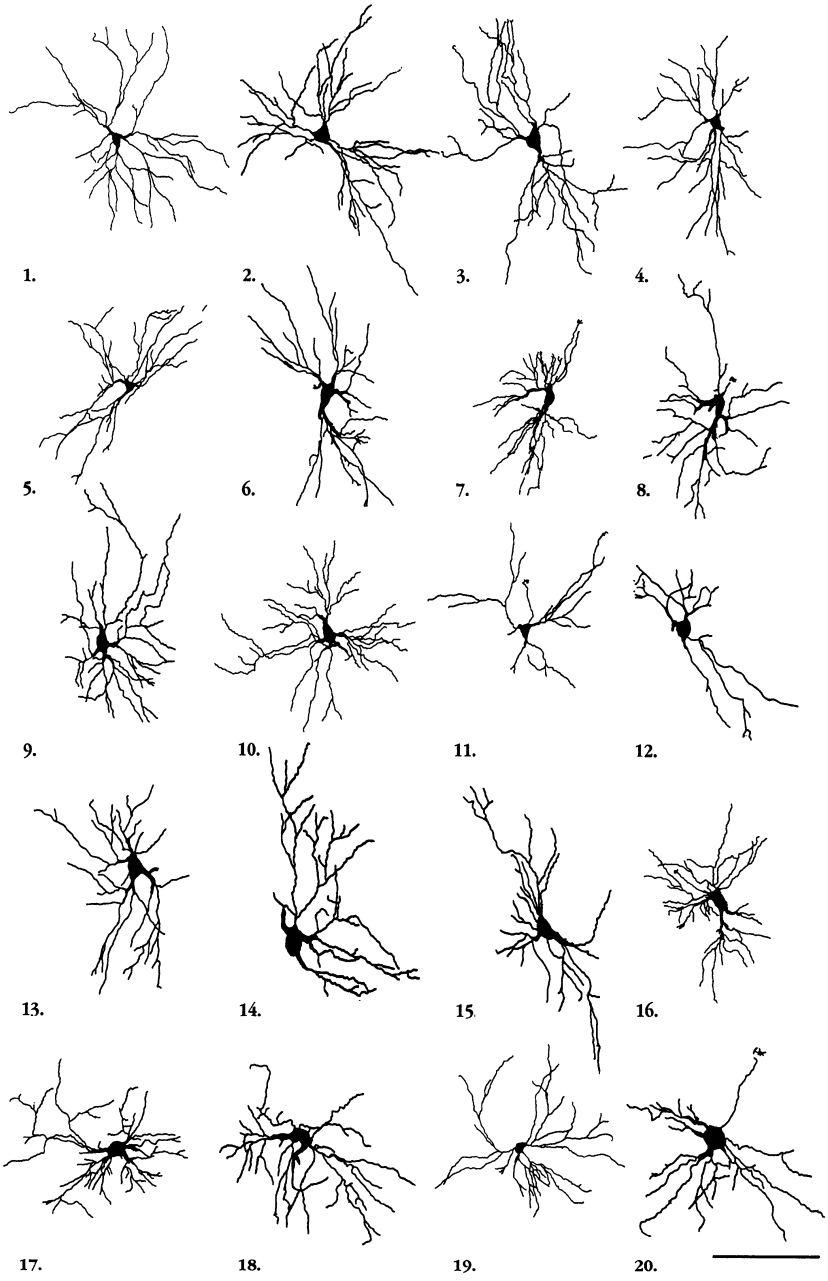

Fig. 4. Dendritic morphology of the neurons of the dorsal thalamic nuclei in camera lucida drawings from rapid Golgi-impregnated sections. The neurons from the LDDM (1–12) have smaller somata and more numerous radially extended dendrites, while the neurons from the LDVL (13–20) in general have larger somata with fewer dendrites. The dendritic arborization of the neurons in these dorsal thalamic nuclei is mostly vertically oriented. Scale bar: 100 μm

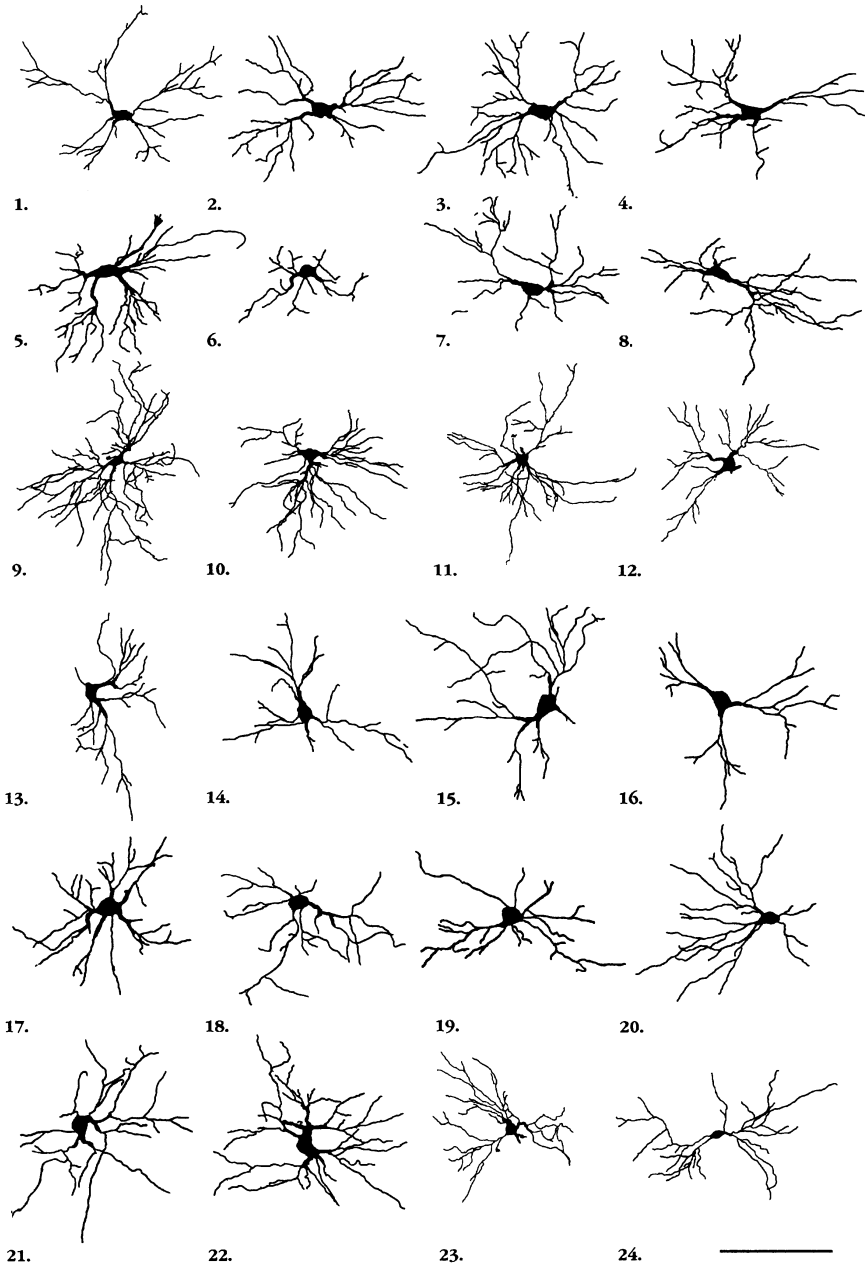

Fig. 5. Dendritic morphology of the neurons of the ventral thalamic nuclei in camera lucida drawings from rapid Golgi-impregnated sections. The dendritic arborization of the VM neurons (1–12) and the VL neurons (13–24) is mostly horizontally oriented. Scale bar: 100 μm

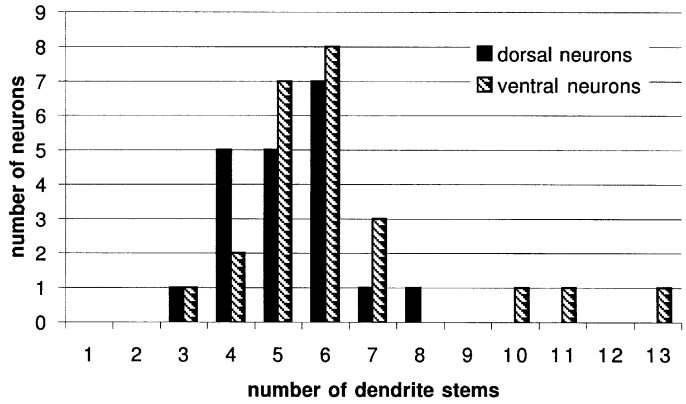

Fig. 6. Quantitation of dendritic stems in the dorsal and ventral thalamic nuclei. The numbers of dendritic stems in the dorsal and ventral nuclei are 3–8 and 3–13, respectively

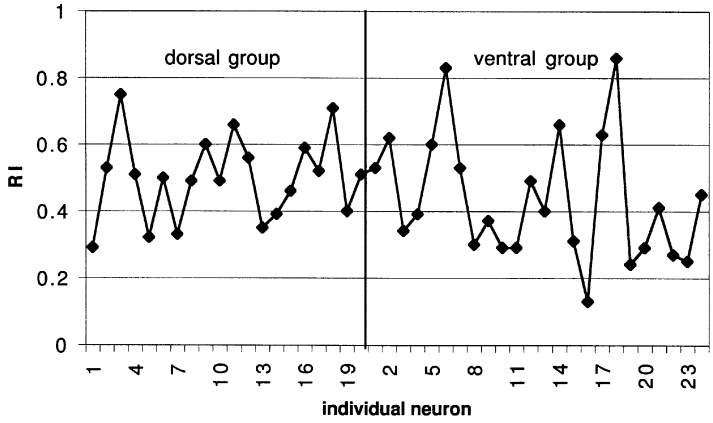

Fig. 7. Distribution of RI values of the neurons analyzed in this study. Of the 44 neurons analyzed, 4 (9%) had significantly larger RI values than the average

from the dorsal and ventral parts of the thalamus were 0.5 and 0.44, respectively (Table 1). These neurons amounted to about 86% of the neurons investigated in this study. It should be noted, however, that about 7% of the total number of neurons examined had RI values close to 0.75. These type A neurons (e.g. dorsal thalamic neuron 3 in Fig. 4 and ventral thalamic neurons 6 and 18 in Fig. 5) had highly-polarized, imbalanced, spatially more asymmetric dendritic arbors. A few type C neurons (about 7%) were also present, with RI values of around 0.25, e.g. neurons 16, 19 and 23 in Fig. 5. A graphical representation of the RI values is to be seen in Fig. 7.

Table 1
Quantitative parameters (S, H and RI) of the neurons from the dorsal and ventral thalamic nuclei

Neuron no.	Height (H)		Size (S)		Relative imbalance (RI)	
	dorsal neurons	ventral neurons	dorsal neurons	ventral neurons	dorsal neurons	ventral neurons
1	5.00	4.60	6.50	4.60	0.29	0.53
2	4.60	4.80	5.16	4.40	0.53	0.62
3	6.00	4.50	6.25	5.30	0.75	0.34
4	4.50	4.20	4.50	4.60	0.51	0.39
5	3.60	3.38	4.16	2.61	0.32	0.60
6	3.87	3.30	3.75	2.30	0.50	0.83
7	4.00	3.60	4.60	3.30	0.33	0.53
8	5.25	4.00	7.00	3.80	0.49	0.30
9	5.16	4.20	5.60	4.60	0.60	0.37
10	4.30	5.20	3.80	6.00	0.49	0.29
11	4.60	5.20	4.30	7.50	0.66	0.29
12	3.80	6.00	3.20	8.30	0.56	0.49
13	4.60	3.50	5.00	3.50	0.36	0.40
14	5.00	3.60	7.00	3.00	0.39	0.66
15	4.40	3.60	4.40	3.30	0.47	0.32
16	4.42	3.20	4.00	3.00	0.59	0.13
17	6.80	3.50	9.80	3.14	0.52	0.63
18	5.00	4.00	5.30	3.20	0.72	0.86
19	6.00	2.70	7.50	2.00	0.40	0.24
20	4.00	3.16	3.50	2.83	0.52	0.29
21		3.00		2.42		0.41
22		2.63		2.27		0.27
23		5.25		6.25		0.25
24		5.00		5.25		0.45
mean:	4.75	4.01	5.27	4.06	0.50	0.44
SD:	0.79	0.87	1.60	1.64	0.12	0.18
t-test:		0.007		0.021		0.222

DISCUSSION

Parameters S and H provide basic quantitative information on the dendritic arborization of a neuron. However, they are insufficient for a full characterization of a dendritic tree. As an example, we demonstrated that, on the basis of these parameters alone, two pairs of thalamic nuclei, LDDM and LDVL in the dorsal part, and VL and VM in the ventral part, differed significantly from each other. Parameters H and S are indicative of the length and the complexity of a dendritic tree, respectively, and they do not give information on the spatial distribution and orientation of the branches. Our data showed that the dendritic arbor could not be characterized reliably purely on the basis of H and S. However, parameter RI depends not only on H and S (direct-

ly in the case of S, or indirectly in the case of H), but also involves the spatial characteristics of the branching pattern of the dendritic tree. When RI was introduced to study the dendritic arborization, and the geometric pattern of the branching was introduced into the analysis, no significant differences were observed between these neuronal groups. Differences in branching pattern in various types of neurons could be of particular interest in studies of pathological situations or spatial interactions of postsynaptic events [6].

In the present study we have extended the number of available parameters to characterize the dendritic arborization. Introduction of parameter RI could be of value in further morphometric studies.

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