

TOPOLOGICAL ANALYSIS OF DENDRITIC GROWTH AT VARIOUS STAGES
OF CEREBRAL DEVELOPMENT

H.B.M. Uylings, R.W.H. Verwer, J. Van Pelt and
J.G. Parnavelas

Netherlands Institute for Brain Research, IJdijk
28, 1095 KJ Amsterdam, The Netherlands and Dept
Cell Biology, Health Sciences Center, Dallas, TX,
USA

ABSTRACT

The value of a new method for the topological analysis of tree structures, developed by Verwer and Van Pelt (1983), is illustrated with the application of observed dendritic branching patterns at various stages of cerebral development. The new classification scheme of topological types facilitates growth hypothesis testing. Now, small sample sizes can be analysed efficiently. The dendritic networks of two major neuron types in the cerebral cortex, viz. pyramidal cells and multipolar non-pyramidal cells were examined to test the terminal and segmental growth hypotheses. The analysis showed that during the various developmental stages the observations on both neuron types were in agreement with the terminal growth predictions.

INTRODUCTION

In this communication, we illustrate some advantages of a new topological method (Van Pelt and Verwer, 1982a,b; Verwer and Van Pelt, 1983) by its application to the dendritic branching patterns of nerve cells at various developmental stages. The questions we examined were (a) which mode of branching occurs in the dendritic growth processes of two major types of cerebral cortical neurons and (b) is the mode of branching subject to changes during the developmental stages of the dendritic trees.

The mode of branching can be inferred from the comparison of the frequencies of observed branching patterns with predicted probabilities. Two particular growth models, i.e. terminal and segmental growth, are used in such diverse topics as neuronal patterns, river patterns and evolutionary trees (e.g. Berry and Bradley, 1976; Smit et al., 1972; Dacey and Krumbein, 1976; Harding, 1971). Terminal growth means that new branches are formed randomly at the terminal segments. Segmental growth implies that new branches originate at random from all segments. The new topological method (cf. Verwer and Van Pelt, 1983) enables the testing of the growth mode of small sample sizes. This may be important since, in practice, sample sizes of dendritic trees are frequently small. Either too many small trees occur in the early developmental stages or histological sectioning produces many incomplete larger trees.

MATERIAL AND METHODS

The developmental stages examined in female Sprague-Dawley rats were days 6, 10, 12, 14, 16, 18, 20 and 90 post partum. In 120 μm thick Golgi-Cox stained sections, the bifurcation patterns of layer II/III pyramidal neurons and layer IV multipolar non-pyramidal neurons (cf. Parnavelas and Uylings, 1980) in the occipital cortex were measured with a semi-automatic tracking system (Overdijk et al., 1978) or from camera lucida drawings with a MOP- AMO2 Kontron digitizer. Twelve to twenty-five neurons of each type per animal were measured, totalling 411 neurons. The neurons were selected in the following manner. In both left and right hemisphere the first neuron measured was located in the specific layer at the medial side of the cortex. Proceeding laterally within the cortical area each neuron encountered and satisfying the selection criteria was measured. The selection criteria comprised; good quality of neuronal staining and the presence of at least two reasonably complete dendrites within the histological section.

Since the topological methods have already been described in detail elsewhere (Van Pelt and Verwer, 1982; Verwer and Van Pelt, 1983) only a short summary will be given here. The number of terminal segments defines the degree of the tree structure. For a specific degree the tree structures can be divided into a number of classes

according to (a) the degrees of the two first-order subtrees and (b) the degrees of all pairs of subtrees. In this paper we used classification (a). The notation of the set of n -th degree trees with r and $n-r$ degree first-order subtrees is $A(r, n-r)$, with $r \leq n-r$. To test the two above-mentioned growth hypotheses, it may be necessary to use a two class division (Verwer and Van Pelt, 1983) when the sample sizes are small. For segmental growth analysis the first class corresponds to $A(1, n-1)$ and the other to

$$\sum_{r=2}^{[n/2]} A(r, n-r), \text{ where } [n/2] \text{ is the integral part of } n/2. \text{ Thus,}$$

for each n the probability of class I under the segmental growth hypothesis, $p_s(r=1|n)$, is defined (see Table 1). To test the terminal growth model the two classes are defined in a different way. Here class I and class II correspond to

$$\sum_{r=1}^m A(r, n-r) \text{ and } \sum_{r=m+1}^{[n/2]} A(r, n-r) \text{ respectively, where } m \text{ is the}$$

integral part of $n/4$. Thus the probability of class I, $p_t(r \leq m|n)$, is defined (see Table 2). The Kolmogorov goodness-of-fit test for discontinuous distributions is preferred for small samples (Conover, 1980).

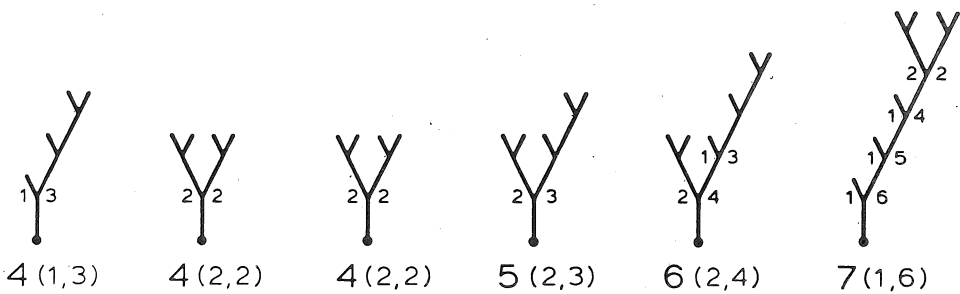


Fig. 1. Observed tree structures with more than 3 terminal segments (tree degree ≥ 4) for day 6 pyramidal cells.

RESULTS

In fig. 1 the six observed complete tree structures for

TABLE 1. SEGMENTAL GROWTH ANALYSIS
(only first-order subtrees for Fig.1 tree patterns)

tree degree	$p_s(r=1 n)$	number of observations	
		class I	class II
4	0.8000	1	2
5	0.7143	-	1
6	0.6667	-	1
7	0.6364	1	-

expected frequency of observations in class I for this sample (μ_s):

$$\mu_s = 1/6 (3 \times 0.8 + 0.7143 + 0.6667 + 0.6364) = 0.7362$$

hypothetical distribution	0.7362	0.2638
observed distribution	2/6	4/6

Kolmogorov test: level of significance 0.045

TABLE 2. TERMINAL GROWTH ANALYSIS
(only first-order subtrees for Fig.1 tree patterns)

tree degree	m	$p_t(r \leq m n)$	number of observations	
			class I	class II
4	1	0.6667	1	2
5	1	0.5000	-	1
6	1	0.4000	-	1
7	1	0.3333	1	-

expected frequency of observations in class I for this sample (μ_t):

$$\mu_t = 1/6 (3 \times 0.6667 + 0.5 + 0.4 + 0.3333) = 0.5389$$

hypothetical distribution	0.5389	0.4611
observed distribution	2/6	4/6

Kolmogorov test: level of significance 0.38 - 0.42

day 6 pyramidal cells are presented. In addition, Tables 1 and 2 show the expected probabilities of class I under both growth hypotheses for the specific degrees of n (ranging from 4 to 7). The expected class I probabilities for the whole sample were calculated, such that hypothesis testing could be performed with the discrete Kolmogorov test. It appeared that the segmental growth hypothesis was rejected and the terminal growth hypothesis could be accepted. All the developmental stages analysed showed a significant rejection of segmental growth irrespective of neuron type (p -value is less than .001 in 8 out of 13 cases). Both for the basal dendrites of the layer II/III pyramidal cells and the dendrites of the layer IV multipolar non-pyramidal cells, the observations for all developmental stages were in agreement with the terminal growth hypothesis (Tables 3 and 4).

 TABLE 3. TERMINAL GROWTH ANALYSIS
 with Kolmogorov goodness-of-fit test for discontinuous
 distribution (only first-order subtrees are incorporated)

AGE (days)	FREQUENCY IN CLASS I		LEVEL OF SIGNIFICANCE
	Hypothetical	Observed	
layer II/III pyramidal cell, basal dendrites			
6	0.5389	2/6	0.38 - 0.42
10	0.5269	18/34	0.75 - 1.00
14	0.4882	33/83	0.10
18	0.4995	12/34	0.12
90	0.5435	13/26	0.58 - 0.70
dendrites of layer IV multipolar non-pyramidal cells			
10	0.5328	16/28	0.59 - 0.71
12	0.5411	17/37	0.30 - 0.33
14	0.5629	19/33	0.75 - 1.00
16	0.5183	25/47	0.69 - 0.88
18	0.5584	21/42	0.46 - 0.53
20	0.5446	16/35	0.29 - 0.33
24	0.5731	14/31	0.19 - 0.20
90	0.5206	18/39	0.45 - 0.52

TABLE 4. SEGMENTAL GROWTH ANALYSIS
with Kolmogorov goodness-of-fit test for discontinuous
distribution (only first-order subtrees are incorporated)

AGE (days)	FREQUENCY IN CLASS I		LEVEL OF SIGNIFICANCE
	Hypothetical	Observed	
layer II/III pyramidal cell, basal dendrites			
6	0.7362	2/6	.045
10	0.7124	17/34	.008
14	0.6874	29/83	<.001
18	0.6966	11/34	<.001
90	0.7088	11/26	.002
dendrites of layer IV multipolar non-pyramidal cells			
10	0.7249	15/28	.033
12	0.7222	16/37	<.001
14	0.7261	17/33	.010
16	0.7071	19/47	<.001
18	0.7297	19/42	<.001
20	0.7291	15/35	<.001
24	0.7524	14/31	<.001
90	0.7232	18/39	<.001

DISCUSSION

Our topological methods can be applied to any group of binary tree networks (like lungs, rivers, axons, dendrites, phylogenetic- and botanical tree structures. The topological classification of trees developed by Verwer and Van Pelt(1982) considerably facilitates the topological analysis and extends the range of tools for morphometric branching pattern analysis (e.g. Uylings et al., 1981). In contrast to previous topological procedures, our method can group together samples of trees with different degrees (i.e. number of terminal segments) in testing a growth hypothesis. Combined with the discrete Kolmogorov test this enables the analysis of small sample sizes, of which we have presented an example. These results, and our unpublished results for the bitufted non-pyramidal dendrites, show that in the

cerebral cortex all the major neuron types are in agreement with the terminal growth model. An exception is the apical dendrite of pyramidal neurons (e.g. Hollingworth and Berry, 1975). Furthermore, our findings suggest that the mode of branching does not change during the development of pyramidal basal dendrites and the dendrites of multipolar non-pyramidal cells.

ACKNOWLEDGEMENTS

The authors thank Carla de Raay, Mary Blue and Anatoli Chatzissavidou for their extensive technical assistance.

REFERENCES

- Berry M, Bradley, PM. The application of network analysis to the study of branching patterns of large dendritic fields. *Brain Res* 1976; 109: 111-32.
- Conover WJ. *Practical Nonparametric Statistics*. New York: Wiley and Sons, 1980: 344-84.
- Dacey MF, Krumbein WC. Three growth models for stream channel networks. *J Geol* 1976; 84: 153-63.
- Harding EF. The probabilities of rooted tree-shapes generated by random bifurcation. *Adv Appl Prob* 1971; 3: 44-77.
- Hollingworth T, Berry M. Network analysis of dendritic fields of pyramidal cells in neocortex and Purkinje cells in the cerebellum of the rat. *Phil Trans Roy Soc Lond B* 1975; 270: 227-64.
- Overdijk J, Uylings HBM, Kuypers K, Kamstra AW. An economical, semiautomatic system for measuring cellular tree structures in three dimensions, with special emphasis on Golgi-impregnated neurons. *J Microsc (Oxford)* 1978; 114: 271-84.
- Parnavelas JG, Uylings HBM. The growth of non-pyramidal neurons in the visual cortex of the rat: A morphometric study. *Brain Res* 1980; 193: 373-82.
- Smit GJ, Uylings HBM, Veldmaat-Wansink L. The branching pattern in dendrites of cortical neurons. *Acta morphol Neerl.-Scand* 1972; 9: 253-74.
- Uylings HBM, Parnavelas JG, Walg HL. Morphometry of cortical dendrites. In: Vidrio EA, Galina MA. eds. *Advances in the Morphology of Cells and Tissues*. *Progr Clin Biol Res* Vol 59. New York: Liss Inc., 1981: 185-92.

- Van Pelt J, Verwer RWH. The exact probabilities of branching patterns under terminal and segmental growth hypotheses. Bull Math Biol 1982; in press.
- Van Pelt J, Verwer RWH. New classification methods of branching patterns. J Microsc (Oxford) 1982; in press.
- Verwer RWH, Van Pelt J. A new method for the topological analysis of neuronal tree structures. J Neurosci Meth 1982; submitted.