

A new method for the topological analysis of neuronal tree structures

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Statistical analysis of the frequencies of observed branching patterns of neuronal arborescences is an important means of studying neuronal growth and of characterizing axonal or dendritic populations. We recently derived simple formulae for the exact probabilities of occurrence of types of neuronal trees for both segmental and terminal growth. Additionally, the existence of a natural ordering of the neuronal tree types enables the application of the Kolmogorov goodness-of-fit test. In the present report it is illustrated how these facilities can be incorporated in the analysis of neuronal arborizations. Interesting features are that very large neuronal arborizations can be analyzed completely and that only small sample sizes are required for the estimation of the critical level corresponding to the growth hypothesis. Further, it is indicated how populations of neuronal tree structures may be compared with each other without reference to a particular growth theory.

Introduction

Developing or regenerating neurons adapt the outgrowth of their dendrites and axons via functional interactions with other cells (Berry et al., 1980). At any stage during the process of outgrowth the result of the inductive interactions is reflected in the geometrical properties of the dendrites or axons. Statistical analysis of these properties contributes much to our understanding of the growth process and may also be used to characterize different cell types (Uylings et al., 1981). This approach to the study of branching processes ignores the metrical parameters and only incorporates topological parameters in the statistical procedure.

Two hypothetical modes of binary branching, i.e. segmental and terminal growth, have received much attention (Smit et al., 1972; Berry et al., 1975; Berry and Bradley, 1976). Segmental growth implies that protrusion of new branches occurs with equal probability from any existing segment. On the other hand, with terminal growth only terminal segments form new branches with equal probability. For arborizations with an equal number of terminal segments it is possible to distinguish different topological types. If for every stage in the growth process a hypothetical

mode of branching is specified, then for the set of trees with a certain degree (i.e. number of terminal segments) the probability distribution of the corresponding types is completely determined. Illustrative examples of both growth processes may be found in a recent review of Berry et al. (1980). In previous neurobiological studies (Smit et al., 1972; Berry et al., 1975; Berry and Bradley, 1976) the probability distributions were determined by computer simulation. In a different context (i.e. phylogeny) Harding (1971) derived the exact probability of each topological type under a hypothesis corresponding to terminal growth. For segmental growth the exact probability of each type was deduced by Van Pelt and Verwer (1983). The number of possible types rapidly increases as the trees become enlarged (cf. Harding, 1971). Therefore it was proposed to classify the possible types such that a significant reduction of the number of classes and a natural ordering of the classes was achieved (Van Pelt and Verwer, 1983).

Comparing observed with hypothetical frequency distributions of neuronal tree types provides insight into the underlying growth process. In the proposed analytical procedure, the comparison is founded on the natural ordering of the classes and the corresponding exact probabilities (Van Pelt and Verwer, 1983). As a consequence of the existing order, the Kolmogorov goodness-of-fit test for discrete distributions may be applied (Conover, 1972; Horn, 1977; Wood and Altavela, 1978; Conover, 1980). The application of this very sensitive test reduces the minimally required number of observations as compared with other goodness-of-fit tests for discrete data (Horn, 1977).

However, the topological analysis of neuronal arborizations is not necessarily restricted to the comparison of hypothetical (i.e. based on growth theories) with observed distributions. For instance, it may be important to compare two or more frequency distributions of observed neuronal tree structures without reference to a particular mode of growth. We will briefly discuss some aspects of this kind of analysis.

Outline of the procedure

1. General

Biological branching structures usually extend in 3 dimensions. When the arborizations have been formed by binary branching, it is possible to determine with the presented method whether one of the above-mentioned growth hypotheses gives a good description of the actual branching process. In Fig. 1 a two-dimensional (2-D) representation of such an arborization is shown. By convention we always display 2-D representations of tree structures such that, at any bifurcation point, the right hand branch is larger (i.e. more terminal segments) than the left one. To each bifurcation point an order is assigned according to the number of segments between that point and the root of the tree. Thus, the first-order bifurcation point is that point at which the root segment bifurcates. The branches originating from the first-order bifurcation point are called first-order subtrees. Higher-order bifurcation

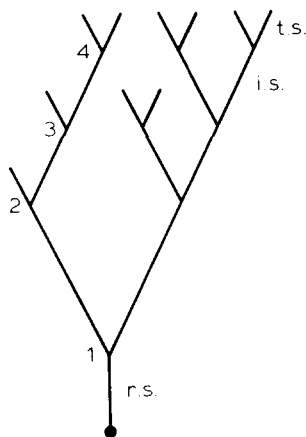


Fig. 1. Example of the 2-D representation of a tree structure. The black dot at the origin of the tree is called the root. The segment emerging from the root is the root segment (r.s.). The other segments are either terminal segments (t.s.) or intermediate segments (i.s.). The numbers at the bifurcation points denote the order (i.e. the topological distance from the root) of these bifurcation points.

points and subtrees are defined in a similar way (Fig. 1). Many different types of tree structures of the same degree are possible and there are several ways to group these types into classes (Van Pelt and Verwer, 1983). For the method presented here it suffices to classify the arborizations according to the degree of the smaller of the two first-order subtrees. For example, if r and $n - r$ ($r \leq n - r$) represent, respectively, the degrees of the left (smallest) and right first-order subtree of a network with n terminal segments, then $A(r, n - r)$ denotes the corresponding class. The resulting classes are arranged according to the value of r (Fig. 2).

2. Comparison of observed and hypothetical distributions of arborizations

2.1. Construction of the cumulative frequency distribution of observed arborizations.

First, the number of terminal segments of each neuronal tree is established. Then, it is determined how this number is partitioned between the first-order subtrees. This division determines the corresponding class, as was mentioned above. After all observed structures, with the same number of terminal segments, have been classified accordingly into classes a cumulative frequency distribution is constructed. An example is presented in Fig. 2, where observed trees of degrees ranging from 4 through 11 have been classified. Under both growth hypotheses the partition of the terminal segments over the subtrees of any bifurcation point is independent of the preceding bifurcations. Thus, each subtree, with more than 3 terminal segments, can be considered as an individual observation. These subtrees might be included in the appropriate classifications of the parent trees.

2.2. Calculation of the hypothetical probability distributions of trees. Van Pelt and Verwer (1983) showed that under the hypothesis of terminal growth an arborization

of degree n has probability

$$p_t(r, n - r) = 2^{1 - \delta_{r, n-r}} \cdot \frac{1}{n - 1} \tag{1}$$

that its first-order subtrees have degree r and $n - r$ respectively. The Kronecker delta-function (δ) is defined as:

$$\begin{aligned} \delta_{r, n-r} &= 0 & \text{if } r &\neq n - r \\ \delta_{r, n-r} &= 1 & \text{if } r &= n - r \end{aligned}$$

From Eqn. 1 it follows that all classes for which $r = n - r$ have equal probability and that the class for which $r = n - r$ (only possible when n is even) has half the probability of each of the other classes. For segmental growth the following expression for the probability that a tree has first-order subtrees of degree r and

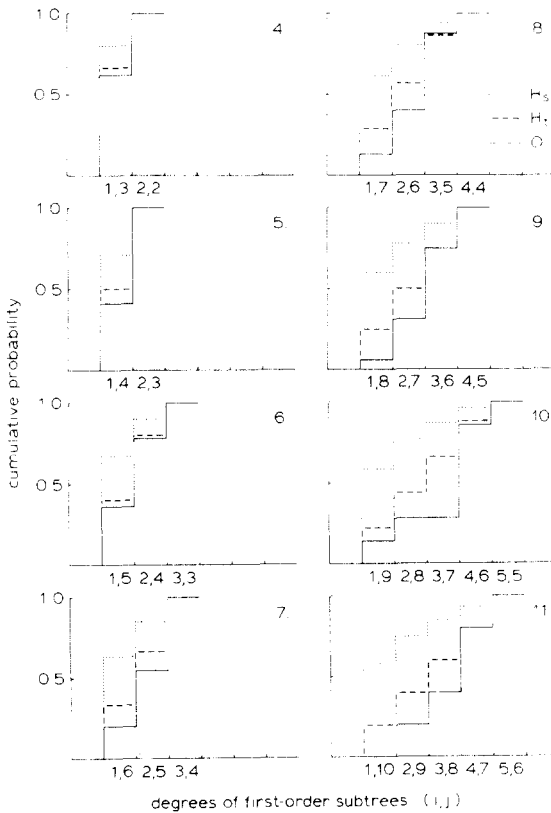


Fig. 2. Cumulative probability distributions of arborizations with 4 through 11 terminal segments according to their first order subtrees. H_s : cumulative distribution under the segmental growth hypothesis, H_t : cumulative distribution under the terminal growth hypothesis and O : the normalized frequency distribution of observed basal dendrites of pyramidal cells.

$n - r$, respectively

$$p_s(r, n - r) = 2^{1 - \delta_{r, n-r}} \cdot \frac{(2n - 1)}{(2r - 1)(2n - 2r - 1)} \cdot \frac{\binom{2r - 1}{r} \times \binom{2n - 2r - 1}{n - r}}{\binom{2n - 1}{n}} \quad (2)$$

was derived (Van Pelt and Verwer, 1983). It appears that for segmental growth the probabilities decrease monotonously when r runs from 1 to its maximal value.

When the cumulative probability distributions have been constructed with these formulae the difference between terminal and segmental growth is evident (Fig. 2). However, to decide whether an observed frequency distribution is compatible with one of the growth hypotheses, statistical analysis is indispensable (Table I).

An interesting feature from a practical point of view is that as the degree approaches infinity, the theoretical distributions converge to two distinct asymptotic distributions. For instance, consider the probability that a tree of degree n has first-order subtrees of degrees 1 and $n - 1$ under segmental growth

$$p_s(1, n - 1) = \frac{2n(n - 1)}{(2n - 2)(2n - 3)} \quad (3)$$

This is apparent from substituting $r = 1$ into Eqn. 2 and it is easily verified that the limit of $p_s(1, n - 1)$ for infinite n is:

$$\lim_{n \rightarrow \infty} p_s(1, n - 1) = \frac{1}{2} \quad (4)$$

Thus, as the size of the trees increases, the probability of observing trees of degree n having first-order subtrees of degrees 1 and $n - 1$ tends to $1/2$ under segmental growth hypothesis (Fig. 3). Similarly, simple calculation shows that for terminal growth the classes 1 through m ($m = \text{int}(n/4)$) of the probability distribution contain about 50% of the observations in a sample of structures of degree n ($n \rightarrow \infty$). By

TABLE I

THE LEVEL OF SIGNIFICANCE OBTAINED BY THE KOLMOGOROV GOODNESS-OF-FIT TEST FOR DISCRETE DISTRIBUTIONS FOR THE DATA SHOWN IN FIG. 2.

No of terminal segments	No of observations	Level of significance under the hypothesis of:	
		terminal growth	segmental growth
4	60	0.43-0.49	8×10^{-4}
5	29	0.41-0.45	7×10^{-4}
6	36	0.73-0.96	2×10^{-4}
7	29	0.27-0.29	3×10^{-6}
8	16	0.30-0.37	2×10^{-4}
9	15	0.33-0.36	1×10^{-5}
10	7	0.09	3×10^{-3}
11	5	0.82-1.00	0.01

definition m is the integral part of $n/4$. Since

$$p_t(r, n - r) = \frac{2}{n - 1} \tag{5}$$

for each $r \neq n - r$, the proportion of structures contained in the first m classes is

$$p_t(r \leq m, n - r) = \frac{2m}{n - 1} \tag{6}$$

Then, as n increases, m tends to $n/4$ because the remainder of $n/4$ is always smaller than 1.

Hence,

$$\lim_{n \rightarrow \infty} p_t(r \leq m, n - r) = \frac{1}{2} \tag{7}$$

Thus, according to the terminal growth hypothesis, the probability of observing a tree, having first-order subtrees with degrees $r \leq n/4$ and $n - r \geq 3n/4$, approaches $1/2$ when the trees become large (Fig. 3).

2.3. *Testing the similarity of an observed and a hypothetical distribution.* After cumulative distributions, based on the first-order bifurcations (cf. Section 2.1), have been constructed for observed trees testing against the growth hypotheses may be performed. Each group of trees, possibly including subtrees, of the same degree is tested separately (Fig. 2 and Table I). Using the Kolmogorov goodness-of-fit test for discrete distributions (Conover, 1972, 1980) the corresponding two-sided level of significance is calculated. If the level of significance is less than a preset ($\alpha = 0.05$) value we reject the hypothesis, otherwise it is accepted (see Results). We apply this analysis only when the number of observed trees with the same degree is at least 5, although the Kolmogorov test does not require this (Conover, 1980). Otherwise, we lump the classes of each cumulative distribution such that two classes result (Fig. 3), and analyse the combination of the different distributions as described below.

When a number of very large trees (degree at least 300) is observed, it is unlikely that they all have the same degree. By making use of the asymptotic approximations (Section 2.2), all observed trees, irrespective of their particular degree, can be combined into one single two-class distribution which is defined differently for each specific growth hypothesis (cf. Fig. 3). One simply counts the number of terminal segments in both first-order subtrees of each network to decide to which class it belongs. The two classes of the asymptotic distributions have been chosen such that 50% of the observations in a sample of sufficiently large trees (i.e. with more than about 300 terminal segments) can be assigned to class I when the null hypothesis is true (Fig. 3). Consequently, the level of significance for a sample of large trees could be obtained from a corresponding binomial test, with $p = 1/2$ (cf. Conover, 1980, pp. 95–99).

Sometimes, it is necessary or useful to analyse together a group of smaller dendrites, many of them having different degrees (degree may range from 4 to 300). In Fig. 4 such a sample of observations is shown. Since the asymptotic approximation cannot be applied we proceed in the following way. After the type of each observed tree is established (cf. Section 2.1), the trees are classified in a two-class

ASYMPTOTIC PROBABILITY DISTRIBUTIONS FOR VERY LARGE ARBORIZATIONS ($n \rightarrow \infty$)

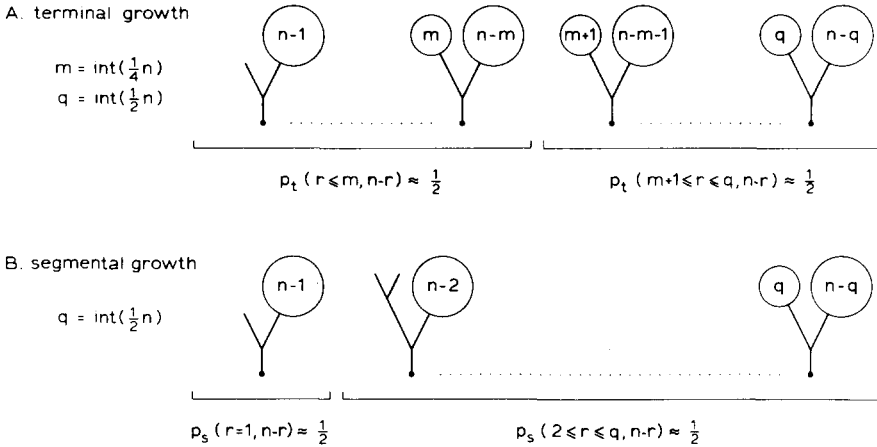


Fig. 3. The lumped classification of topological tree types for terminal and segmental growth hypothesis for the analysis of combined distributions. N.B. This lumping scheme may also be applied to combine trees of smaller degree. Then, however, the probability that a tree belongs to class I is not 1/2, but depends on the corresponding degree.

distribution, according to the same criteria as used with the asymptotic approximation (see Fig. 3). However, instead of a constant probability ($p = 1/2$), we have variable probabilities, depending on the degrees of the trees. The probability that an observed tree belongs to class I (cf. Fig. 3) must be calculated from Eqns. 3 and 6 for segmental growth and terminal growth, respectively. Values of these probabilities for trees starting with degree 4 through 300 are presented in the Appendix (Tables A1 and A2). For a particular sample of trees, the number of observations, that are expected to be assigned to class I, is equal to the sum of the probabilities that individual trees, based on their degree, belong to class I. Dividing this sum by the

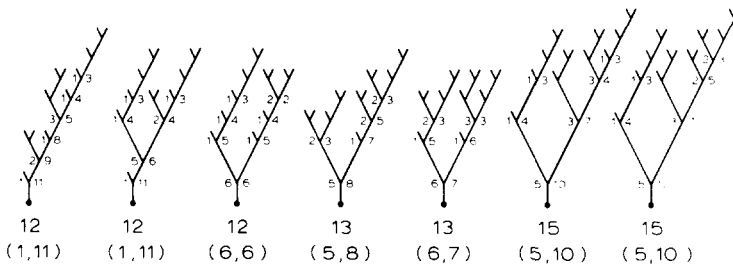


Fig. 4. Example of observed pyramidal dendritic tree types for the analysis of combined distributions. The observed trees are presented in relative position according to the arrangement described by Van Pelt and Verwer (1983). The numbers surrounding each bifurcation point show the partition of the terminal segments among the two subtrees.

total number of observations yields the expectation (mean probability) $\mu(\mu_1 \text{ or } \mu_2)$ that any of the observed trees belong to class I, when the null hypothesis is true (cf. Results). The expectation that any tree belongs to class II is $1 - \mu$. Subsequently, for each growth hypothesis, the distribution of observed trees is tested with the discrete Kolmogorov test, or χ^2 test when the number of observations is large, against the two-class distribution with theoretical probabilities μ and $1 - \mu$. This way of testing is likely to be somewhat conservative, since the test ignores the fact that the probability μ is the average of variable probabilities. Namely, the variance of μ appears to be maximal when the probabilities that constitute μ are all equal (Hoeffding, 1956; Feller, 1970, pp. 230–231). As was mentioned before subtrees might well be included and it is therefore possible to test one single large tree against a growth hypothesis, when its subtrees are used to calculate μ as indicated above.

3. Comparison of two or more observed distributions of neuronal arborizations

To compare two or more experimental distributions a k-sample test (or equivalently the χ^2 -test for differences in probabilities; cf. Conover, 1980, p. 222), or a k-sample Smirnov test (Conover, 1980, p. 333) can be applied (see also the discussion). We did not include examples of these tests with observations because the applications are straightforward.

Experimental

1. Materials

The data used to illustrate our approach were kindly provided by Dr. H.B.M. Uylings from our institute. The measurements have been performed on Golgi-impregnated pyramidal cells from layer III of the visual cortex of adult Wistar rats. Dendritic trees that were not completely binary have been deleted from the samples. The semi-automatic tracking device used to obtain the data has been described elsewhere (Overdijk et al., 1978).

A DIGITAL VAX 11/780 computer was used for the processing of the data. In the Kolmogorov goodness-of-fit test for discrete distributions, intermediate results in the calculations can reach excessive values, such that the precision of the computer (determined by the number of bits per data item) imposes restrictions on the maximum number of observations (Conover, 1972; Horn, 1977). In our experience, for calculations with double precision floating point numbers, the limit is about 90 observations. It may be noted that the computations for the Kolmogorov test can be satisfactorily performed on a programmable desk-calculator (one of the authors formerly used a Hewlett-Packard 9825 A calculator for this test) provided that the number of observations is less than about 40. The Kolmogorov test for discrete distributions can be applied to large samples (Wood and Altavela, 1978), however, the complicated procedure involves Monte Carlo simulations and most neurobiologists might prefer to apply the χ^2 -test in such cases.

2. Results

Observations of dendrites of pyramidal neurons of adult rats were processed. Samples of trees with degree less than 12 were used for the initial analysis. In Fig. 2 the observed frequencies for trees with 4 through 11 terminal segments are compared with the hypothetical distributions. It is evident that the observed distributions are closer to the terminal distribution than to the segmental distribution. When we applied the Kolmogorov test the terminal growth hypothesis was in good agreement

TABLE II

SUMMARY OF THE TEST PROCEDURE FOR COMBINATIONS OF DISTRIBUTIONS BASED ON THE OBSERVATIONS IN FIG. 4.

Only first-order bifurcations have been incorporated. For terminal growth the number of observations in class I corresponds to the number of trees having first-order subtrees r and $n-r$, with $r \leq m$. The corresponding hypothetical probability is $p_t(r \leq m)$ and μ_t is the average of $p_t(r \leq m)$ for different n . For segmental growth class I corresponds to trees having first-order subtrees $(1, n-1)$ and $p_s(1, n-1)$ and μ_s are the hypothetical and average probability respectively.

TERMINAL GROWTH

Tree type	m	$p_t(r \leq m)$	Number of observations	
			class I	class II
12(1, 11)	3	0.54545	2	–
12(6, 6)	3	0.54545	–	1
13(5, 8)	3	0.50000	–	1
13(6, 7)	3	0.50000	–	1
15(5, 10)	3	0.42857	–	2

$$\mu_t = \frac{1}{7} \times (3 \times 0.54545 + 2 \times 0.50000 + 2 \times 0.42857) = 0.49907$$

Hypothetical distribution 0.49907 0.50093

Observed distribution 2/7 5/7

Kolmogorov test: $d_{\max} = 0.213$ Level of significance: 0.40–0.45

SEGMENTAL GROWTH

Tree type	$p_s(1, n-1)$	Number of observations	
		class I	class II
12(1, 11)	0.57143	2	–
12(6, 6)	0.57143	–	1
13(5, 8)	0.56522	–	1
13(6, 7)	0.56522	–	1
15(5, 10)	0.55556	–	2

$$\mu_s = \frac{1}{7} \times (3 \times 0.57143 + 2 \times 0.56522 + 2 \times 0.55556) = 0.56512$$

Hypothetical distribution 0.56512 0.42488

Observed distribution 2/7 5/7

Kolmogorov test: $d_{\max} = 0.279$ Level of significance: 0.24–0.25

TABLE III
SUMMARIZED TEST PROCEDURE FOR COMBINED DISTRIBUTIONS OF OBSERVATIONS FROM FIG. 4 INCLUDING BIFURCATIONS OF ANY ORDER

Symbols have the same meaning as in Table II, while μ_t and μ_s have been calculated analogously to those in Table II

OBSERVED (SUB)TREE TYPES					
Tree types	Number of observations	Tree type	Number of observations	Tree type	Number of observations
4(1, 3)	7	7(1, 6)	1	11(2, 9)	1
4(2, 2)	1	7(2, 5)	2	11(5, 6)	1
5(1, 4)	6	7(3, 4)	1	12(1, 11)	2
5(2, 3)	3	8(1, 7)	1	12(6, 6)	1
6(1, 5)	3	8(3, 5)	1	13(5, 8)	1
6(2, 4)	1	9(1, 8)	1	13(6, 7)	1
6(3, 3)	1	10(3, 7)	2	15(5, 10)	2

TERMINAL GROWTH				SEGMENTAL GROWTH			
n	m	$p_t(r \leq m)$	Number of observations		$p_s(1, n - 1)$	Number of observations	
			class I	class II		class I	class II
4	1	0.66667	7	1	0.80000	7	1
5	1	0.50000	6	3	0.71429	6	3
6	1	0.40000	3	2	0.66667	3	2
7	1	0.33333	1	3	0.63636	1	3
8	2	0.57143	1	1	0.61539	1	1
9	2	0.50000	1	-	0.60000	1	-
10	2	0.44444	-	2	0.58824	-	2
11	2	0.40000	1	1	0.57895	-	2
12	3	0.54545	2	1	0.57143	2	1
13	3	0.50000	-	2	0.56522	-	2
15	3	0.42857	-	2	0.55556	-	2

$\mu_t = 0.49313$	$\mu_s = 0.66737$
Kolmogorov test: $d_{\max} = 0.0319$	$d_{\max} = 0.1674$
Level of significance: 0.61-0.75	Level of significance: 2.9×10^{-2}

with the observations, whereas segmental growth was unanimously rejected (Table I). To illustrate the application of the analysis to combinations of distributions we used samples of trees with degrees 12, 13 and 15, each of which contained too few data to be analysed individually (see Fig. 4). The test procedure, in which only first-order bifurcations are incorporated, is summarized in Table II. To test against terminal growth we obtained for each tree the probability of occurrence in class I from Table A1 of the Appendix. For instance, a tree of degree 12 has a probability of 0.54545 to be of type 12(1, 11), 12(2, 10) or 12(3, 9) (i.e. class I) and a probability

0.45455 to be of type 12(4, 8), 12(5, 7) or 12(6, 6) (i.e. class II). By taking the average of these probabilities for all observed trees it was determined that for the sample under consideration 49.907% of the observed trees would be expected to be in class I. Two of the seven trees actually belonged to class I, which resulted in acceptance of the terminal growth hypothesis. In the same way (using Table A2 of the Appendix) it was found that segmental growth could not be rejected either. Therefore, we could not discriminate between terminal and segmental growth on the basis of these 7 observations. We increased the number of observations by including the higher-order bifurcations in the analysis (compare Fig. 4 and Table III). The additional information leads to rejection of the segmental growth hypothesis and an even better agreement with terminal growth.

Discussion

A new approach to the analysis of the growth of neuronal trees has been described. This approach has some noteworthy advantages. Contrary to previously published applications of binary-network analysis (Smit et al., 1972; Berry et al., 1975; Berry and Bradley, 1976) we incorporated exact expressions for the hypothetical probability distributions. Comparison between terminal and segmental growth by means of cumulative probability distributions based on grouped arborizations reveals a striking difference (cf. Fig. 2). It appears that terminal growth has a nearly uniform distribution. In contrast, the distribution for segmental growth is highly skewed, such that the probability increases as the size difference between the two first-order subtrees of an arborization increases. The agreement of experimental data with each of the hypothetical distributions can be assessed by the Kolmogorov goodness-of-fit test for discontinuous distributions (Conover, 1972; Conover, 1980). Contrary to the χ^2 -test, the Kolmogorov test takes the natural order of the classes into account and, therefore, the latter is more powerful than the former (Horn, 1977). As a consequence of the application of the discrete Kolmogorov test, only few (5 or more) observations are required and large dendrites may be analysed. With large samples (more than about 90 observations) one might apply the χ^2 -test but additional lumping may be necessary to ensure that the expected number of observations in each class is large enough (Horn, 1977; Conover, 1980, p. 191). It may happen that discrimination between the growth hypotheses cannot be made on basis of an observed sample, then, additional observations must be obtained. This can be accomplished either by inclusion of higher-order bifurcations (e.g. subtrees of degree 4 from larger arborizations can be added to the distribution of trees of degree 4, etc.) or by measuring more trees.

A different situation occurs when one wants to analyse trees and/or subtrees of different degree together (N.B. the subtrees may all belong to one large tree). Two classes are defined according to the specific growth model (cf. Fig. 3) and for each observed (sub-) tree, the probability to belong to class I, is used to calculate the mean proportion (μ) of (sub-) trees expected to be in class I (cf. Tables II and III). The actually observed proportion of (sub-) trees in class I is compared with the expected proportion.

If more than about 5 very large networks (degree larger than 300) are observed, it may be sufficient to scan quickly the partitioning of the terminal segments over the two first-order subtrees of each network and use the asymptotic approximation. The corresponding level of significance can be determined easily from a table of binomial distributions (Conover, 1980. p. 222 and Table A3).

When the cumulative distributions of the observed dendritic trees of superficial pyramidal neurons were compared with the theoretical distributions for terminal and segmental growth (Fig. 2) it appeared that the distributions of terminal growth and the observed data were very close. This was confirmed when the Kolmogorov goodness-of-fit test for discontinuous distributions was applied (Table I). Agreement with terminal growth was also found when the discrete Kolmogorov test was applied to the analysis of the combined distribution of trees with degrees 12, 13 and 15 (Tables II and III). In order to avoid misinterpretations it must be remembered that distributions are compared and that an actual growth process, albeit different from the hypothesized one, may lead to similar probabilities. In this respect it is worth mentioning that, in spite of the agreement with terminal growth, a tendency, that the trees are more symmetric, can be discerned from the data in the examples (Fig. 2). Whether this has biological significance is a point for further studies. Still, the proposed analysis can be very helpful to find alternative modes of growth when systematic deviations from the hypothetical growth models are found. Although we have presented examples of dendritic trees only, axonal arborizations may very well be analysed similarly.

In addition, it may be interesting to check whether axons or dendrites show different branching patterns, when they develop under different conditions. Likewise, it may be necessary to study the similarity of the branching patterns of two or more populations of dendrites or axons. In such cases the k-sample χ^2 -test (Conover, 1980) may be the most appropriate to apply. With respect to the existence of a natural order, the application of the k-sample Smirnov test (Noether, 1967; Conover, 1980) might be considered. Although the Smirnov test assumes continuously distributed data, Noether (1967) suggested that it might be worthwhile to apply this test to discrete distributions when the number of observations is sufficiently large. However, in those cases that we compared these two tests the k-sample Smirnov test appeared even more conservative than the k-sample χ^2 -test.

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Appendix

In this Appendix the probabilities corresponding to class I of the two-class distribution in Fig. 3 for terminal and segmental growth are presented. The tables contain data for trees with degree (n) ranging

from 4 through 300. In Table A1 m denotes the number of classes that constitute the new class I, whereas the remaining classes constitute class II. Then $p(r \leq m)$ denotes the probability that an observed tree belongs to any of the classes $A(1, n-1), \dots, A(m, n-m)$. In Table A2 $p(1, n-r)$ refers to the probability that an observed tree belongs to class $A(1, n-1)$.

TABLE A1

TERMINAL GROWTH PROBABILITIES FOR FIRST "m" CLASSES

n	m	$p(r \leq m)$	n	m	$p(r \leq m)$	n	m	$p(r \leq m)$
1	0	-	52	12	0.480000	101	25	0.500000
2	0	-	52	13	0.509804	102	25	0.495050
3	0	-	53	13	0.500000	103	25	0.490196
4	1	0.666667	54	13	0.490566	104	26	0.504854
5	1	0.500000	55	13	0.481481	105	26	0.500000
6	1	0.400000	56	14	0.509091	106	26	0.495238
7	1	0.333333	57	14	0.500000	107	26	0.490566
8	2	0.571429	58	14	0.491228	108	27	0.504673
9	2	0.500000	59	14	0.482759	109	27	0.500000
10	2	0.444444	60	15	0.508475	110	27	0.495413
11	2	0.400000	61	15	0.500000	111	27	0.490909
12	3	0.545455	62	15	0.491803	112	28	0.504505
13	3	0.500000	63	15	0.483871	113	28	0.500000
14	3	0.461538	64	16	0.507937	114	28	0.495575
15	3	0.428571	65	16	0.500000	115	28	0.491228
16	4	0.533333	66	16	0.492308	116	29	0.504348
17	4	0.500000	67	16	0.484848	117	29	0.500000
18	4	0.470588	68	17	0.507463	118	29	0.495726
19	4	0.444444	69	17	0.500000	119	29	0.491525
20	5	0.526316	70	17	0.492754	120	30	0.504202
21	5	0.500000	71	17	0.485714	121	30	0.500000
22	5	0.476190	72	18	0.507042	122	30	0.495868
23	5	0.454545	73	18	0.500000	123	30	0.491803
24	6	0.521739	74	18	0.493151	124	31	0.504065
25	6	0.500000	75	18	0.486486	125	31	0.500000
26	6	0.480000	76	19	0.506667	126	31	0.496000
27	6	0.461538	77	19	0.500000	127	31	0.492063
28	7	0.518519	78	19	0.493506	128	32	0.503937
29	7	0.500000	79	19	0.487179	129	32	0.500000
30	7	0.482759	80	20	0.506329	130	32	0.496124
31	7	0.466667	81	20	0.500000	131	32	0.492308
32	8	0.516129	82	20	0.493827	132	33	0.503817
33	8	0.500000	83	20	0.487805	133	33	0.500000
34	8	0.484848	84	21	0.506024	134	33	0.496241
35	8	0.470588	85	21	0.500000	135	33	0.492537
36	9	0.514286	86	21	0.494118	136	34	0.503704
37	9	0.500000	87	21	0.488372	137	34	0.500000
38	9	0.486486	88	22	0.505747	138	34	0.496350
39	9	0.473684	89	22	0.500000	139	34	0.492754
40	10	0.512821	90	22	0.494382	140	35	0.503597
41	10	0.500000	91	22	0.488889	141	35	0.500000
42	10	0.487805	92	23	0.505495	142	35	0.496454
43	10	0.476190	93	23	0.500000	143	35	0.492958

TABLE A1 (continued)

n	m	$p(r \leq m)$	n	m	$p(r \leq m)$	n	m	$p(r \leq m)$
44	11	0.511628	94	23	0.494624	144	36	0.503497
45	11	0.500000	95	23	0.489362	145	36	0.500000
46	11	0.488889	96	24	0.505263	146	36	0.496552
47	11	0.478261	97	24	0.500000	147	36	0.493151
48	12	0.510638	98	24	0.494845	148	37	0.503401
49	12	0.500000	99	24	0.489796	149	37	0.500000
50	12	0.489796	100	25	0.505051	150	37	0.496644
151	37	0.493333	201	50	0.500000	251	62	0.496000
152	38	0.503311	202	50	0.497512	252	63	0.501992
153	38	0.500000	203	50	0.495050	253	63	0.500000
154	38	0.496732	204	51	0.502463	254	63	0.498024
155	38	0.493506	205	51	0.500000	255	63	0.496063
156	39	0.503226	206	51	0.497561	256	64	0.501961
157	39	0.500000	207	51	0.495146	257	64	0.500000
158	39	0.496815	208	52	0.502415	258	64	0.498054
159	39	0.493671	209	52	0.500000	259	64	0.496124
160	40	0.503145	210	52	0.497608	260	65	0.501931
161	40	0.500000	211	52	0.495238	261	65	0.500000
162	40	0.496894	212	53	0.502370	262	65	0.498084
163	40	0.493827	213	53	0.500000	263	65	0.496183
164	41	0.503067	214	53	0.497653	264	66	0.501901
165	41	0.500000	215	53	0.495327	265	66	0.500000
166	41	0.496970	216	54	0.502326	266	66	0.498113
167	41	0.493976	217	54	0.500000	267	66	0.496241
168	42	0.502994	218	54	0.497696	268	67	0.501873
169	42	0.500000	219	54	0.495413	269	67	0.500000
170	42	0.497041	220	55	0.502283	270	67	0.498141
171	42	0.494118	221	55	0.500000	271	67	0.496296
172	43	0.502924	222	55	0.497738	272	68	0.501845
173	43	0.500000	223	55	0.495495	273	68	0.500000
174	43	0.497110	224	56	0.502242	274	68	0.498168
175	43	0.494253	225	56	0.500000	275	68	0.496350
176	44	0.502857	226	56	0.497778	276	69	0.501818
177	44	0.500000	227	56	0.495575	277	69	0.500000
178	44	0.497175	228	57	0.502203	278	69	0.498195
179	44	0.494382	229	57	0.500000	279	69	0.496403
180	45	0.502793	230	57	0.497817	280	70	0.501792
181	45	0.500000	231	57	0.495652	281	70	0.500000
182	45	0.497238	232	58	0.502165	282	70	0.498221
183	45	0.494505	233	58	0.500000	283	70	0.496454
184	46	0.502732	234	58	0.497854	284	71	0.501767
185	46	0.500000	235	58	0.495726	285	71	0.500000
186	46	0.497297	236	59	0.502128	286	71	0.498246
187	46	0.494624	237	59	0.500000	287	71	0.496503
188	47	0.502674	238	59	0.497890	288	72	0.501742
189	47	0.500000	239	59	0.495798	289	72	0.500000
190	47	0.497354	240	60	0.502092	290	72	0.498270
191	47	0.494737	241	60	0.500000	291	72	0.496552
192	48	0.502618	242	60	0.497925	292	73	0.501718
193	48	0.500000	243	60	0.495868	293	73	0.500000

TABLE A1 (continued)

n	m	$p(r \leq m)$	n	m	$p(r \leq m)$	n	m	$p(r \leq m)$
194	48	0.497409	244	61	0.502058	294	73	0.498294
195	48	0.494845	245	61	0.500000	295	73	0.496599
196	49	0.502564	246	61	0.497959	296	74	0.501695
197	49	0.500000	247	61	0.495935	297	74	0.500000
198	49	0.497462	248	62	0.502024	298	74	0.498316
199	49	0.494949	249	62	0.500000	299	74	0.496644
200	50	0.502513	250	62	0.497992	300	75	0.501672

TABLE A2

SEGMENTAL GROWTH PROBABILITIES FOR THE FIRST CLASS

n	$p(1, n-1)$	n	$p(1, n-1)$	n	$p(1, n-1)$
1	-	51	0.515152	101	0.507538
2	-	52	0.514851	102	0.507463
3	-	53	0.514563	103	0.507389
4	0.800000	54	0.514286	104	0.507317
5	0.714286	55	0.514019	105	0.507246
6	0.666667	56	0.513761	106	0.507177
7	0.636364	57	0.513514	107	0.507109
8	0.615385	58	0.513274	108	0.507042
9	0.600000	59	0.513043	109	0.506977
10	0.588235	60	0.512821	110	0.506912
11	0.578947	61	0.512605	111	0.506849
12	0.571429	62	0.512397	112	0.506787
13	0.565217	63	0.512195	113	0.506726
14	0.560000	64	0.512000	114	0.506667
15	0.555556	65	0.511811	115	0.506608
16	0.551724	66	0.511628	116	0.506550
17	0.548387	67	0.511450	117	0.506494
18	0.545455	68	0.511278	118	0.506438
19	0.542857	69	0.511111	119	0.506383
20	0.540541	70	0.510949	120	0.506329
21	0.538462	71	0.510791	121	0.506276
22	0.536585	72	0.510638	122	0.506224
23	0.534884	73	0.510490	123	0.506173
24	0.533333	74	0.510345	124	0.506122
25	0.531915	75	0.510204	125	0.506073
26	0.530612	76	0.510067	126	0.506024
27	0.529412	77	0.509934	127	0.505976
28	0.528302	78	0.509804	128	0.505929
29	0.527273	79	0.509677	129	0.505882
30	0.526316	80	0.509554	130	0.505837
31	0.525424	81	0.509434	131	0.505792
32	0.524590	82	0.509317	132	0.505747
33	0.523810	83	0.509202	133	0.505703
34	0.523077	84	0.509091	134	0.505660
35	0.522388	85	0.508982	135	0.505618

TABLE A2 (continued)

n	p(1, n - 1)	n	p(1, n - 1)	n	p(1, n - 1)
36	0.521739	86	0.508876	136	0.505576
37	0.521127	87	0.508772	137	0.505535
38	0.520548	88	0.508671	138	0.505495
39	0.520000	89	0.508571	139	0.505455
40	0.519481	90	0.508475	140	0.505415
41	0.518987	91	0.508380	141	0.505376
42	0.518519	92	0.508287	142	0.505338
43	0.518072	93	0.508197	143	0.505300
44	0.517647	94	0.508108	144	0.505263
45	0.517241	95	0.508021	145	0.505226
46	0.516854	96	0.507937	146	0.505190
47	0.516484	97	0.507853	147	0.505155
48	0.516129	98	0.507772	148	0.505119
49	0.515789	99	0.507692	149	0.505085
50	0.515464	100	0.507614	150	0.505051
151	0.505017	201	0.503759	251	0.503006
152	0.504983	202	0.503741	252	0.502994
153	0.504950	203	0.503722	253	0.502982
154	0.504918	204	0.503704	254	0.502970
155	0.504886	205	0.503686	255	0.502959
156	0.504854	206	0.503667	256	0.502947
157	0.504823	207	0.503650	257	0.502935
158	0.504792	208	0.503632	258	0.502924
159	0.504762	209	0.503614	259	0.502913
160	0.504732	210	0.503597	260	0.502901
161	0.504702	211	0.503580	261	0.502890
162	0.504673	212	0.503563	262	0.502879
163	0.504644	213	0.503546	263	0.502868
164	0.504615	214	0.503529	264	0.502857
165	0.504587	214	0.503513	265	0.502846
166	0.504559	216	0.503497	266	0.502836
167	0.504532	217	0.503480	267	0.502825
168	0.504505	218	0.503464	268	0.501814
169	0.504478	219	0.503448	269	0.502804
170	0.504451	220	0.503432	270	0.502793
171	0.504425	221	0.503417	271	0.502783
172	0.504399	222	0.503401	272	0.502773
173	0.504373	223	0.503386	273	0.502762
174	0.504348	224	0.503371	274	0.502752
175	0.504323	225	0.503356	275	0.502742
176	0.504298	226	0.503341	276	0.502732
177	0.504274	227	0.503326	277	0.502722
178	0.504249	228	0.503311	278	0.502712
179	0.504225	229	0.503297	279	0.502703
180	0.504202	230	0.503282	280	0.502693
181	0.504178	231	0.503268	281	0.502683
182	0.504155	232	0.503254	282	0.502674
183	0.504132	233	0.503240	283	0.502664
184	0.504110	234	0.503226	284	0.502655
185	0.504087	235	0.503212	285	0.502646

TABLE A2 (continued)

n	p(1, n - 1)	n	p(1, n - 1)	n	p(1, n - 1)
186	0.504065	236	0.503198	286	0.502636
187	0.504043	237	0.503185	287	0.502627
188	0.504021	238	0.503171	288	0.502618
189	0.504000	239	0.503158	289	0.502609
190	0.503979	240	0.503145	290	0.502600
191	0.503958	241	0.503132	291	0.502591
192	0.503937	242	0.503119	292	0.502582
193	0.503916	243	0.503106	293	0.502573
194	0.503896	244	0.503093	294	0.502564
195	0.503876	245	0.503080	295	0.502555
196	0.503856	246	0.503067	296	0.502547
197	0.503836	247	0.503055	297	0.502538
198	0.503817	248	0.503043	298	0.502530
199	0.503797	249	0.503030	299	0.502521
200	0.503778	250	0.503018	300	0.502513

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