

ANALYSIS OF BINARY TREES WHEN OCCASIONAL MULTIFURCATIONS CAN BE CONSIDERED AS AGGREGATES OF BIFURCATIONS

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The geometrical properties of neurons are important for the way they function within neural circuits. The arborescent processes of neurons that are necessary for the transmission of the information are formed by branching and elongation of segments. In studies that model the outgrowth the tree structures have generally been considered as binary. However, multifurcations do occur. It will be shown that if the multifurcations can be considered as aggregates of bifurcations they may be included in the topological analysis of neuronal branching patterns.

Introduction. The shape of neurons is largely determined by their dendritic processes. The diversity of neuronal forms suggests that this property is important for the particular functioning of the neurons. Incoming signals on the dendritic trees are integrated and lead to a response via the axon. Likewise, the shape of axonal branching patterns is relevant for the innervation of a particular brain region. The formation of the shape of dendritic and axonal branching patterns is regulated partly by genetic factors and partly by interactions with the environment (Woodward *et al.*, 1977; Berry *et al.*, 1981; Eysel *et al.*, 1985; Purves and Lichtman, 1985; Coates, 1986; Cohan and Kater, 1986). Although all the members of a given type of neurons have the same shape, large variations both in metrical and topological parameters do occur. Thus, the formation of a particular tree structure is subjected to stochastic fluctuations of either intrinsic or extrinsic nature, or both. This implies that studies dedicated to describe the probabilistic nature of branching events may be useful. For more than a decade, the branching process of neuronal tree structures has been studied using topological growth models (Smit *et al.*, 1972; Berry *et al.*, 1975; Hollingworth and Berry, 1975; Sadler and Berry, 1983; Van Pelt and Verwer, 1983; 1985; 1986; Van Pelt *et al.*, 1986; Horsfield *et al.*, 1987; Kliemann, 1987). In these growth models simple rules are defined for the binary branching process that ultimately results in a variety of tree shapes as also observed in nature. Each event in the branching process consists of the selection of a new branching point along some existing segment in the tree. The

new branching point divides the original segment into two new segments, which may be of unequal length. Moreover, a new terminal segment emanates from the new branching point. Growth models can be tested with the so-called subtree partition analysis (cf. Verwer and Van Pelt, 1983; Verwer *et al.*, 1987). Each branching point corresponds to a partition which indicates how the terminal segments or branching points are divided over the subtrees of that branching point. The probability distribution of the partitions is unique for each growth model (Van Pelt and Verwer, 1985; 1986). Another method, which uses the number of subtrees with two terminal segments in a sample of trees to infer the mode of growth, is called vertex analysis (cf. Berry and Flinn, 1984; Verwer *et al.*, 1985). For infinitely large tree structures a different approach to growth modelling has been worked out in which several segments can simultaneously form new branches (Horsfield *et al.*, 1987). This approach was called synchronous growth by Horsfield *et al.* (1987) to distinguish it from the so-called sequential growth which permits only one segment to form a new segment during each growth step. For instance, from this point of view, the models (which include the so called terminal growth and segmental growth models) studied earlier (cf. Berry *et al.*, 1975; Hollingworth and Berry, 1975; Van Pelt and Verwer, 1985; 1986) are sequential. The synchronous growth model is, at present, only applicable to infinitely large trees. The above-mentioned growth models and analytical methods assume that neuronal trees are binary. This means that two subtrees should emanate from each branching point to enable analysis in terms of these growth models. However, natural trees may contain a certain amount of multifurcations, depending on the type of neuron. Berry *et al.* (1986) noted that up to 10% of the branching points may be multifurcations in some neuronal trees. The majority of these are trifurcations. Two ways in which a multifurcation may be formed are; (1) a number of bifurcations emerge at very short distance of each other (Fig. 1A), or, (2) more than one new segment emanate from the new branching point during a branching event (Fig. 1B). Recently, we have studied growth models for the formation of multifurcations for the second possibility (Verwer and Van Pelt, 1987). These models are straightforward extensions of the growth models we have derived before (Van Pelt and Verwer, 1985; 1986). For tree structures whose branching points all have the same multiplicity (i.e. the same number of subtrees arises from each branching point) the models can be formulated analytically. However, when the branching points have variable multiplicity (e.g. bifurcations, trifurcations and maybe even higher multifurcations occur simultaneously) the branching process can only be described by computer simulations. Berry *et al.* (1986) also used computer simulations to generate trees with varying percentages of trifurcations to study the effect on vertex analysis. If multifurcations originate inherently in the growth process (cf. Fig. 1B), the binary models are of course not appropriate to study this process.

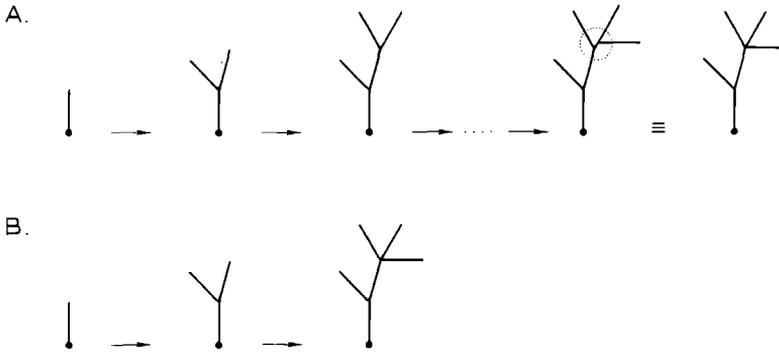


Figure 1. Schematic diagram illustrating two possible alternative branching processes, that could lead to the observation of a trifurcation. (A) First a bifurcation is produced and at some later moment very close to the existing branching point a new bifurcation event takes place. This trifurcation may be considered as an aggregate of bifurcations. (B) Apart from the formation of bifurcations, sometimes three segments arise simultaneously from one branching point. Such a trifurcation can only be regarded as a 'real' trifurcation.

If, alternatively, multifurcations are observed because of limited spatial resolution the binary growth models (both sequential and synchronous) remain applicable to trees containing such multifurcations (cf. Fig. 1A). For instance, the distance between two or more adjacent bifurcation points may be small with respect to the diameter of the branches. An example of an event as described by Fig. 1A may be found in Fig. 3 of Bray and Bunge (1973). As a result we actually have an aggregate of bifurcations, whose precise sequence cannot be observed. Indeed, neurobiologists generally treat multifurcations in dendrites or axons as if they occur due to limited resolution rather than as a consequence of the growth process (cf. Glaser *et al.*, 1979; Percheron, 1979; Calvet and Calvet, 1984; Berry *et al.*, 1986; Verwer and Van Pelt, 1986). In the rest of this paper we will assume that neuronal trees are essentially binary and occasional multifurcations should be considered as aggregates of bifurcations. In a previous study (Verwer and Van Pelt, 1985), we discussed growth analysis of essentially binary trees containing aggregates of bifurcations. We noticed that the main problem was the classification in terms of bifurcations of the observed multifurcations. More explicitly, it is not known how the observed multifurcations should be assigned to the existing bipartition classes (cf. the first column of Table 1). For example, which class in the first column of Table 1 should contain the 4-furcation $6(1, 1, 2, 2)$? Below, it will be shown that if the classification scheme for bipartitions is extended to contain classes for multipartitions (cf. the other columns in Table 1), an elementary rule from probability theory enables the inclusion of multifurcations in subtree partition analysis. Further, we will discuss the statistical implications of the occurrence of multifurcations in binary trees. The presented solution does not apply

directly to vertex analysis, but in principle it could be used via the probabilities of partitions and rather complex statistical manipulations.

Classification of Multipartitions. To be able to classify a multipartition we need to specify a sequence of all different partitions of the same kind (see below and Figs 2B and 2C). As noted in the introduction, a partition indicates how the number of terminal segments (degree) is subdivided over the subtrees that arise from a particular branching point. In a partition the subtrees are considered as equivalent if they have the same number of terminal segments. We will consider partitions to be of the same kind if they have the same number of subtrees (multiplicity) and the same total number of terminal segments (degree) (cf. Figs 2B and 2C). The number of distinguishable partitions $q(n, m)$ of degree (n) and multiplicity (m) can be enumerated by the following recurrent relation:

$$q(n, m) = \sum_{j=1}^m q(n-m, j) \quad (1)$$

with $q(n, m) = 1$ if $n \leq m + 1$ and $q(n, m) = 0$ if $n < m$ and $q(n, m) = 1$ if $m = 1$ (cf. Todd, 1944; Comtet, 1974; Biggs, 1985). Repeated application of relation (1) induces a classification of these partitions (cf. Table 1), which is completely compatible with the classification we have proposed for binary partitions (cf. Van Pelt and Verwer, 1983). Table 1 illustrates the classification if we tabulate the degree of the partitions vertically and the multiplicity horizontally.

Probability of an Aggregate of Bifurcations. In the growth models we have studied up till now, all branching events in a tree are assumed to occur independently of each other. As a consequence, the branching points or the corresponding partitions may be considered as mutually independent observations. The study of tree topology by means of partition analysis is based on the frequencies of occurrence of the partitions in the observed trees. The growth models enable the prediction of the frequencies of binary partitions and the analysis involves comparison of the observed frequencies with the predicted ones. If multifurcations are aggregates of bifurcations the binary growth models are still valid. However, the separate bifurcations, that constitute the multifurcation, cannot be distinguished. Thus, a multifurcation of m subtrees, say, represents $m - 1$ observations (bifurcations), which in fact cannot be observed. These $m - 1$ missing values are substituted by one actual observation (i.e. the multifurcation). This remaining observation can be used in topological analysis of growth models if its probability of occurrence can be determined. An elementary rule from probability theory states that, for any set of mutually exclusive events, the probability of at least one occurring equals the sum of the

Table 1. List of the possible partitions of n terminal segments over m subtrees (for $n \leq 8$ and $m \leq 6$). The multiplicity (m) indicates the number of subtrees emanating from a particular branching point and the degree (n) denotes the number of terminal segments that are divided among these subtrees. It may be noted that Fig. 2A is one of the possible trees that is represented by the third partition with the degree 7 and multiplicity 2. Likewise, Fig. 2D corresponds to the second partition with degree 6 and multiplicity 3

Degree (n)	Multiplicity (m)				
	2	3	4	5	6
2	(1, 1)				
3	(1, 2)	(1, 1, 1)			
4	(1, 3)	(1, 1, 2)	(1, 1, 1, 1)		
	(2, 2)				
5	(1, 4)	(1, 1, 3)	(1, 1, 1, 2)	(1, 1, 1, 1, 1)	
	(2, 3)	(1, 2, 2)			
6	(1, 5)	(1, 1, 4)	(1, 1, 1, 3)	(1, 1, 1, 1, 2)	(1, 1, 1, 1, 1, 1)
	(2, 4)	(1, 2, 3)	(1, 1, 2, 2)		
	(3, 3)	(2, 2, 2)			
7	(1, 6)	(1, 1, 5)	(1, 1, 1, 4)	(1, 1, 1, 1, 3)	(1, 1, 1, 1, 1, 2)
	(2, 5)	(1, 2, 4)	(1, 1, 2, 3)	(1, 1, 1, 2, 2)	
	(3, 4)	(1, 3, 3)	(1, 2, 2, 2)		
		(2, 2, 3)			
8	(1, 7)	(1, 1, 6)	(1, 1, 1, 5)	(1, 1, 1, 1, 4)	(1, 1, 1, 1, 1, 3)
	(2, 6)	(1, 2, 5)	(1, 1, 2, 4)	(1, 1, 1, 2, 3)	(1, 1, 1, 1, 2, 2)
	(3, 5)	(1, 3, 4)	(1, 1, 3, 3)	(1, 1, 2, 2, 2)	
	(4, 4)	(2, 2, 4)	(1, 2, 2, 3)		
		(2, 3, 3)			

probabilities of occurrence of the separate events. In this case each event corresponds to a possible arrangement of bifurcations which in its turn can be considered as a binary tree with m differently labelled leaves. To illustrate the idea of labelled leaves a trifurcation whose subtrees have been marked by labels (a), (b) and (c) is shown in Fig. 2F. This labelled tree might, for instance, represent tree 2D or tree 2E. Note that for tree 2D label (a) corresponds to the subtree with 1 terminal segment, label (b) to the subtree with 2 terminal segments and label (c) to the subtree with 3 terminal segments. In tree 2E the labels (a), (b) and (c) would correspond to 2, 3 and 4 terminal segments. Each subtree in a multifurcation receives a unique label, even if some subtrees may have the same number of terminal segments. Let the labels be the terminal tips of a labelled binary tree, then it is possible to distinguish a number of different types (cf. Fig. 3). The number of labelled binary trees with m labels and therefore the number of possible arrangements of m subtrees over the bifurcations that constitute an aggregate is:

$$N = \frac{(2m-2)!}{2^{m-1} \cdot (m-1)!} \quad (2)$$

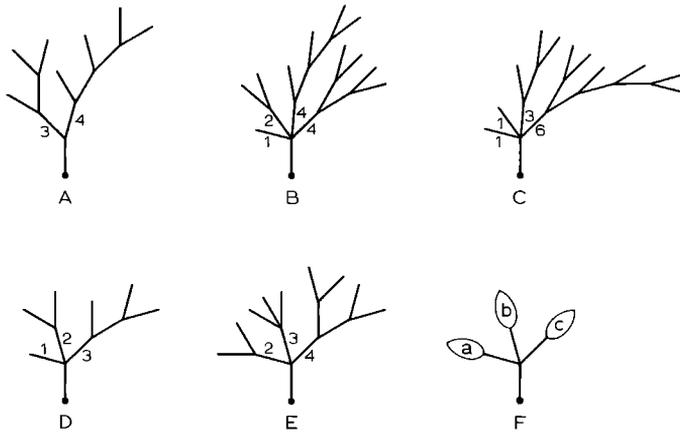


Figure 2. Illustration of the topological aspects of trees that are investigated in this report. (A) Shown is the partition of the terminal segments over the two subtrees of the first bifurcation point of the tree. (B) A tree with a multifurcation whose multiplicity is four (4-furcation). Note that two subtrees have the same number of terminal segments and, therefore, are equivalent, but they do not have an identical shape. The partition of this multifurcation is (1, 2, 4, 4). (C) A multifurcation that is of the same kind as the one in B. They have the same number of subtrees and the same total number of terminal segments. (D) A trifurcation. (E) Another trifurcation, with a subtree also containing a trifurcation. Note that these two trifurcations are independent under the conditions of the growth models discussed in this paper. (F) A labelled trifurcation with three leaves, which could represent both 2D and 2E.

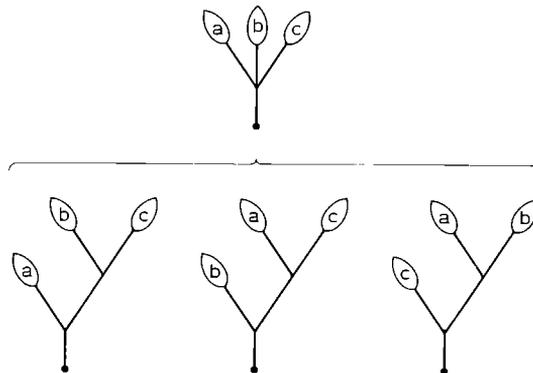


Figure 3. Illustration of the possible binary arrangements of subtrees that could result in the trifurcation at the top by aggregation of two adjacent bifurcations. There are 3 labelled binary trees as follows from equation (2). Note, that each subtree is labelled differently, even if some subtrees are identical.

(cf. Harding, 1971). All N possible arrangements must first be identified and their probabilities must be evaluated. For this purpose the probabilities of the binary partitions of each arrangement are calculated according to a growth model. The (Q, S) -growth models derived by Van Pelt and Verwer (1985; 1986) might be used or the so-called complete partition randomness (cf. Verwer *et al.*, 1987), or any other conceivable model that can be described in terms of partition probabilities. If the binary partitions in each arrangement are independent, as in the case of (Q, S) -models and complete partition randomness (CPR), the probability of each arrangement is the product of the probabilities of its binary partitions. An example of a trifurcation may serve to illustrate the procedure. In Fig. 3 it is shown how the subtrees of a trifurcation might be arranged with two adjacent bifurcations. This is a very simple situation as may be seen from formula (2), because there are only three possible arrangements. Let θ be the parameter set defining the growth model tested and let $n = n_1 + n_2 + n_3$, then:

$$\begin{aligned} h(n_1, n_2, n_3; \theta) = & p(n_1, n_2 + n_3; \theta) p(n_2, n_3; \theta) \\ & + p(n_2, n_1 + n_3; \theta) p(n_1, n_3; \theta) \\ & + p(n_3, n_1 + n_2; \theta) p(n_1, n_2; \theta), \end{aligned} \quad (3)$$

is the unnormalized probability of the aggregate (n_1, n_2, n_3) . To avoid confusion between labels and number of terminal segments we have used a, b and c instead of 1, 2 and 3 in Figs 2F and 3. Let $H(n, 3; \theta)$ be the sum of the unnormalized probabilities for all $q(n, 3)$ possible partitions of n terminal segments over 3 subtrees. Then, the normalized probability $p(n_1, n_2, n_3; \theta)$ is obtained as follows:

$$p(n_1, n_2, n_3; \theta) = \frac{h(n_1, n_2, n_3; \theta)}{H(n, 3; \theta)}. \quad (4)$$

It should be realized that $p(n_1, \dots, n_m; \theta)$ is the probability that an observed multifurcation of degree n and multiplicity m , has a partition (n_1, \dots, n_m) , if the tree has actually grown according to a binary growth model defined by the parameter set (θ) .

Statistical Considerations. Based on the scheme shown in Table 1, each observation, either bifurcation or multifurcation, can be classified. The corresponding expected frequency of occurrence can be calculated using relation (4). It should be noted that the probabilities of the partitions within each block of Table 1 sum to 1, due to the fact that they are conditional on degree and multiplicity. In principle, this would be sufficient for statistical analysis in terms of growth models. The chi-square test might be used to test the

hypothesis that a certain set of observed trees has grown according to a growth model defined by the parameter set θ (cf. Verwer *et al.*, 1987). In practice, the number of observations (i.e. partitions) is seldom very large, which requires that, for both the chi-square test and the minimum chi-square estimation, data are lumped to ensure a sufficient number of expected observations in each class (c.f. Conover, 1980). This lumping can be fairly arbitrary, although it should be remembered that with the selection of the classes and calculation of the expected frequencies, partition probabilities are conditional on the degree and the multiplicity. For instance, if a partition of degree, n , and multiplicity, m , is observed, the probabilities of all possible partitions of this degree and multiplicity must be determined. These possible partitions are subsequently grouped into classes and the probability corresponding to each class can be found by summing the appropriate partition probabilities. The observed partition is assigned to the appropriate class in the frequency distribution of observations. This may be illustrated by an example of the topological analysis of the retinotectal axons in the peripheral tectum of the goldfish. The data were taken from camera lucida drawings in an article of Stuermer (1984). The sample consists of 9 trees containing 151 bifurcations and 9 trifurcations that are topologically relevant. A partition is topologically relevant if its probability is not equal to either 0 or 1. In a previous paper we used the bifurcations from this sample to illustrate the application of maximum likelihood and minimum chi-square estimation to the Q -models (Verwer *et al.*, 1987). The maximum likelihood estimate of the parameter Q for the 151 bifurcations appeared to be 0.4150 with the corresponding level of significance being 0.51. It may be noted that the parameter set θ in this case consists of Q only. The observed trifurcations are listed in Table 2. The partitions 3(1, 1, 1) and 4(1, 1, 2) are irrelevant in the present case, since $p(1, 1, 1; Q) = p(1, 1, 2; Q) = 1$ for each Q . In Fig. 3, it is shown from which sequences of bifurcations the aggregate 6(1, 2, 3) might have been formed (cf. Figs 2D and 2F). The evaluation of relations (3) and (4) for the partitions of degree 6 and multiplicity 3 with $Q = 0.4150$ proceeds as follows:

$$\begin{aligned} h(1, 1, 4; Q) &= 2p(1, 5; Q) p(1, 4; Q) + p(2, 4; Q) p(1, 1; Q) \\ &= 2 \times 0.6172 \times 0.6736 + 0.2699 \times 1.0 = 1.1014, \end{aligned}$$

$$\begin{aligned} h(1, 2, 3; Q) &= p(1, 5; Q) p(2, 3; Q) + p(2, 4; Q) p(1, 3; Q) \\ &\quad + p(3, 3; Q) p(1, 2; Q) \\ &= 0.6172 \times 0.3264 + 0.2699 \times 0.7737 + 0.1128 \times 1.0 = 0.5231, \end{aligned}$$

$$\begin{aligned} h(2, 2, 2; Q) &= 3p(2, 4; Q) p(2, 2; Q) \\ &= 3 \times 0.2699 \times 0.2263 = 0.1832. \end{aligned}$$

Table 2. Example of observed trifurcations in nine retinotectal axonal trees from the goldfish peripheral tectum. The data were collected from camera lucida drawings in Fig. 14 of Stuermer (1984). An observation is assigned to class I if the partition has two subtrees of degree 1. The expected number of the trifurcations has been calculated for $Q=0.415$.

Degree	Partition	Observations			
		Number	Degree	Partition	Number
3	(1, 1, 1)	4	9	(1, 3, 5)	1
4	(1, 1, 2)	1	11	(2, 3, 6)	1
5	(1, 2, 2)	1	19	(1, 8, 10)	1
6	(1, 2, 3)	1	25	(1, 5, 19)	1
7	(2, 2, 3)	1	28	(1, 6, 21)	1
8	(1, 1, 6)	1			

Degree	Observations		Expected number of observations ($Q=0.415$)	
	Class I	Class II	Class I	Class II
5	—	1	0.6296	0.3704
6	—	1	0.6093	0.3907
7	—	1	0.4787	0.5213
8	1	—	0.4781	0.5219
9	—	1	0.4162	0.5838
11	—	1	0.3819	0.6181
19	—	1	0.3256	0.6744
25	—	1	0.3090	0.6910
28	—	1	0.3069	0.6931
Sum	1	8	3.9353	5.0647

The chi-square test statistic: $T=3.89$ with 1 degree-of-freedom.
Level-of-significance: $p=0.05$.

Summing these unnormalised probabilities we find that $H(6, 3; Q)=1.8077$ and thus:

$$p(1, 1, 4; Q)=0.6093,$$

$$p(1, 2, 3; Q)=0.2893,$$

$$p(2, 2, 2; Q)=0.1014.$$

This procedure was repeated for each observed trifurcation. For testing it was decided to lump such that each partition with two subtrees consisting of only 1 terminal segment (i.e. (1, 1, $n-2$)) was assigned to class I and all other partitions were assigned to class II. A similar way of lumping was used before with bifurcations (cf. Verwer and Van Pelt, 1983). Hence, the probability to observe a trifurcation of degree 6 belonging to class I is 0.6093 (see Table 2). The lumping procedure assigned one observation to class I and eight to class II, whereas the expected frequencies were 3.9353 and 5.0647, respectively.

Subsequent testing with the chi-square test showed that the level-of-significance was 0.049. We may conclude that there are fewer partitions of the type $(1, 1, n-2)$, where $n > 4$, than would be expected based on the analysis of bifurcations alone. Application of the minimum chi-square procedure to the combined sample of bifurcations and trifurcations gave an optimal Q -value of 0.39. The corresponding level-of-significance was 0.09 (2 degrees-of-freedom). It should be noted that rejection of a previously accepted growth model due to the incorporation of multifurcations does not imply that the presented method as such is not applicable. Another growth model might be necessary to explain the data.

As mentioned before, the selection of a lumping criterion is rather arbitrary with the chi-square test. But it seems sensible to avoid a very low expected frequency for any class (cf. p. 158 of Conover, 1980). Further, it is desired to test as critically as possible, without making interpretation impossible. The most likely value of the parameter (set) θ for an observed sample can be inferred by applying the chi-square test for different parameter values and selecting that value of θ for which the chi-square statistic is minimal (cf. Verwer *et al.*, 1987). Application of the maximum likelihood procedure involves numerical differentiation, because the terms:

$$\frac{d}{d\theta} \log p(n_1, \dots, n_m; \theta),$$

cannot be evaluated analytically.

Conclusions. In growth model studies of neuronal trees it is normally assumed that branching occurs by random protrusion of a new segment from an already existing one or by random bifurcation of the tips of terminal segments. These assumptions are based on observations from axons *in vivo* (examples may be found in Speidel, 1942; 1964 and in Bray, 1973 and Bray and Bunge, 1973). However, in some types of neurons a substantial number of branching points have a multiplicity greater than 2 (cf. Berry *et al.*, 1986). Speidel (1942) and Bray (1973) found examples of subsequent branching events that occurred at almost the same location on the tree (see also Bray and Bunge, 1973). Whether the respective bifurcation points exactly coincide or that this only appears to be the case due to limited spatial resolution is merely an academic question. The important point here is that the observed trifurcation arose by accident, as an aggregate of bifurcations, and not as a consequence of branching rules inherent in the growth model. This supports the assumption that observed multifurcations in axons or dendrites may actually be considered as aggregates of bifurcations. It must be emphasized that this assumption is crucial for the analytical procedure advanced in this report. If there is evidence that a significant proportion of multifurcations in a certain type of neuron

arises inherently in the growth process, the analysis should be modified accordingly.

Often, it is impossible to investigate the outgrowth of dendrites or axons in a longitudinal study. Therefore, we need to infer this process from once-only observations using growth models. Inherent in the growth models that we have studied so far was that the branching points within any tree are independent, which implies that each branching point can be used as an observation. Since a m -furcation (i.e. a multifurcation with m subtrees) is considered as an aggregate of $m - 1$ indistinguishable bifurcations, such a branching point implies the loss of $m - 1$ observations for which one new observation is returned. We have shown above how the retained information may be used for topological analysis with respect to binary growth models. The efforts needed to accomplish this depend on the degree and the multiplicity of the observed multifurcations [cf. equations (1) and (2) and Table 1]. For instance, there are 85 different trifurcations of degree 28 each of which has three possible arrangements that should be evaluated to calculate the probability of any such trifurcation. At any occasion the decision whether multifurcations will be incorporated in the topological analysis of neuronal tree structures depends on the evaluation of the amount of information that is gained by incorporating them and the cost needed to extract the information from the data. In general, a computer may be required to do the calculations. If the observed multifurcations only consist of trifurcations the computer program is very simple. The majority of multifurcations observed in neuronal trees consists of trifurcations, which suggests that our solution will be easily applicable to most practical situations. It may be noted that our analysis applies to all growth models in which the branching events are mutually independent bifurcations.

Berry *et al.* (1986) have proposed two procedures to deal with trifurcations in neuronal trees. One procedure consists of transforming the particular trifurcations into bifurcations in an arbitrary but systematic fashion. This procedure was only studied for the particular branching process called terminal growth, which corresponds to a value of the aforementioned parameter ($\theta = Q = 0$). Implicit in this growth mode and the transformation procedure is the assumption that trifurcations are aggregates of bifurcations. The other involves comparing equivalent order distributions of both bifurcations and trifurcations. It is not yet clear for either solution how subsequent statistical analysis of observed data should be performed.

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