12 An introduction to topological analysis of neurones

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12.1 Introduction

The nervous system is a natural information processing system, whose components are neurones. The efferent (axons) and afferent (dendrites) elements of neurones carry the signals from one cell to another via specialized contacts (synapses). It is assumed that these connections are specific. The axons and dendrites form arborizations which are important for their functioning in terms of possible contacts and electrical properties. The arborizations of axons and dendrites of a certain class of neurones have a typical shape but they are not identical. The development of shape of the arbors is determined partly by genetic factors and partly by interactions with the surrounding tissue (cf. Levinthal et al. 1975; Woodward et al. 1977; Banker and Cowan 1979; Berry et al. 1981; Easter and Stuermer 1984; Eysel et al. 1985; Haydon et al. 1985; Purves and Lichtman 1985; Hillman 1988; Lasek 1988). Other influences on neuronal tree shape comprise pathological malformations (cf. Jagadha and Becker 1989) and hormonal changes (cf. DeVoogd and Nottebohm 1981; Kurz et al. 1986). These considerations indicate that it would be interesting if we could describe and analyse the shape of neuronal trees. The shape of branching patterns has both metrical and topological aspects. In this review we will focus on descriptors of topological aspects of neuronal tree shape and considerations concerning their application. First, the nature and dynamics of trees, in so far as they are important for our further discussion, will be treated briefly. Then, a number of topological measures and labels with their properties are presented. Further, the comparison of tree data and explanation of the branching process using models will be discussed. The final section contains a few concluding remarks and some suggestions about topics that might be interesting to study in relation to the presented methodology. For recent reviews of metrical aspects we refer to Bjaalie (Chapter 11) and Uylings et al. (1989b).

12.2 The nature of trees

In Fig. 12.1 the camera lucida drawing of a neocortical pyramidal cell together with a schematic representation is shown. The dendrites are branching objects in three-dimensional space having a tortuous appearance. Restricting our attention to topological
Fig. 12.1 (A) A camera lucida drawing of a pyramidal neurone from rat neocortex, stained according to the Golgi–Kopsch procedure. After dehydration the section (500 μm) was left in cedar oil for several hours to enhance its translucency. (B) A schematic drawing of the neurone in (A). The apical dendrite (incomplete) has been labelled with (a) and the basal dendrites have been numbered (1–5) from left to right. Note that the original and schematic dendritic trees are topologically identical. The topological types corresponding to dendrites 1, 2, 3 and 5 can be found in Fig. 12.5. Likewise, the type of dendrite 3 and 4 can be found in Fig. 12.6 as number 6 of degree 6 and number 10 of degree 7 respectively. The circle indicates a trifurcation; whether it is a real trifurcation or an aggregate cannot be inferred from (A).

Properties involves a drastic abstraction of the structure (cf. Fig. 12.1B). The passage from the real tree structure (Fig. 12.1A) to the abstract representation (Fig. 12.1B) involves the reduction of the diameters of branches to zero. In this way we obtain a construction of lines (segments) and points (branching points). We consider only how the segments are connected to each other; the arbitrarily chosen segment length and branching angles in Fig. 12.1B are meaningless. The variability of topological properties can be related to important aspects of morphological variability induced by developmental processes. An impression of the topological variability may be obtained from the dendritic trees of the cell in Fig. 12.1 (see also Fig. 12.9). The concept of tree topology stems from graph theory, but we often deviate from the usual mathematical terminology in order to keep as close as possible to well-known neurobiological terminology (cf. Fig. 12.2). For some mathematical background information on trees we refer to Tucker (1980), Biggs (1985) and Grimaldi (1985). MacDonald (1983) briefly discusses many interesting features of networks and tree-like structures. We will mention a few features that are essential for understanding this review. The elements of a topological tree are points (vertices) and segments (edges), and our interest concerns how these elements are assembled. In a rooted tree we distinguish the root point where the tree starts (for dendrites this is the place where it leaves the soma), branching points (internal vertices) where several segments converge, and terminal tips (external vertices). An intermediate segment connects two branching points and a terminal segment connects a branching point and a terminal tip. At most branching points only two daughter segments arise from the parent segment. Such branching points are called bifurcations (cf. Fig. 12.2A). If a tree contains only bifurcations it is a binary
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Fig. 12.2 Elementary topological properties of trees. (A) Illustration of tree elements. (B) From a multifurcation several subtrees emanate. (C) The degree of a tree or subtree is the number of terminal segments. (D) A first-order partition of the degrees of two subtrees. (E) The partition of a multifurcation. (F) Centrifugal ordering of segments, branching points and terminal tips. Reproduced from Verwer and van Pelt (1986) with permission.

Tree (Biggs 1985; Grimaldi 1985). In general, if from each parent segment \( m \) daughter segments originate we speak of an \( m \)-ary tree. In \( m \)-ary trees the relation between the number of branching points (\( b \)) and terminal segments (\( n \)) is

\[
n = (m-1)b + 1
\]  

(cf. Tucker 1980), while \( m = 2 \) for binary trees. Further, the number of intermediate segments is equal to the number of branching points and the number of terminal segments is equal to the number of terminal tips. Terminal tips are also called leaves (Biggs 1985) and it can be helpful to use this concept when we want to consider some parts of the tree separately (see also Section 12.7).

Neuronal tree structures are often binary; however, some classes of neurones contain a proportion of branching points from which more than two daughter segments emanate (multifurcations; Fig. 12.2B). For instance, retinotectal ganglion cell axons
in goldfish display about 6% trifurcations (cf. Fig. 14 in Stuermer 1984) and Purkinje cell dendrites may contain as much as 10% trifurcations (Hollingworth and Berry 1975). As mentioned above the process of abstraction involves the reduction of a structure extending in three dimensions to a zero-dimensional construction. When several segments converge at some part of a tree (cf. the encircled part of Fig. 12.1B) we are trapped in an ambiguous situation by the abstraction procedure. That is, we must decide whether the segments converge in one point or in a few very closely located points. Thus, an investigator may be unable to decide whether a real multifurcation or a number of very closely located bifurcations is observed. In the latter case it is typically impossible to distinguish the precise sequence of the adjacent bifurcations. This problem of visual resolution depends on such factors as the diameters of the converging segments and the size of the branching ‘point’. An uncertain observation is taken to be a ‘multifurcation’, or it must be ignored. If we wish to analyse data it is necessary to make complementary assumptions concerning the origin of the multifurcations (see Sections 12.3, 12.5 and 12.6). Multifurcations with more than three daughter segments are also observed but with much lower frequencies. In the majority of the methods that describe and analyse tree structures it is assumed implicitly that the trees are strictly binary. Data that are not in agreement with this assumption should be treated by methods that are adapted accordingly. Neuronal trees are observed in whole-mount preparations or in sections after staining. Some observed trees may be incomplete, due to cutting, overlying contamination, or incomplete staining. The corresponding loss of information generally has serious consequences for the analysis (cf. Verwer and van Pelt 1986). The effect of cutting can be minimized and sometimes eliminated by reconstructing the trees from serial sections (Capowski 1989) or by using very thick sections, say 300–700 μm (cf. Glaser and van der Loos 1981).

12.3 Dynamics of trees

In this section a brief account of the dynamical processes that occur in neuronal tree structures is given. Some arguments in Sections 12.5 and 12.6 rely on the observations presented here. At some stages of development neurones start to send out growth cones. As the growth cones follow their course through the environment they establish behind them a segment. At certain moments some growth cone may split up into two or perhaps more parts, which proceed separately to form new segments (Bray 1973). It has been suggested that the splitting of a growth cone depends on targets (synapses) that allow the anchoring of the newly formed segments, thus stabilizing the branching point (cf. Vaughn 1989). If the density of such targets is high then a high frequency of branching would occur (Berry et al. 1981). Others (cf. Banker and Cowan 1979; Banker and Waxman 1988) argue that neurones can generate quite normal dendritic arborizations in such homogeneous environments as culture dishes. According to Bray (1973), sometimes part of a growth cone may be left behind along a segment or at a branching point, while the rest continues to search the environment. At a later stage the part left behind may form a collateral branch. Another observation was that a part of the growth cone climbed back along a fibre (Bray 1973). Apparently, most
growth cones divide into two daughters yielding predominantly binary trees, but we have mentioned before that multifurcations can be found in dendrites and axons. In Fig. 12.3 we show three alternative ways in which, for example, a trifurcation might arise. First, a growth cone might divide into three parts all advancing simultaneously, thereby forming three daughter segments (Fig. 12.3A). This is a real trifurcation. Second, a growth cone may protrude from an already existing segment very close to a branching point (Fig. 12.3B). Actually, the resulting trifurcation is an aggregate of two subsequent bifurcations. Such events have been shown to occur occasionally (cf. Speidel 1942, 1964; Bray 1973; Bray and Bunge 1973). Third, a growth cone might divide into two parts, one continuing on its way, the other dividing again very close to the first branching point (Fig. 12.3C). This is also an aggregate of bifurcations. As far as we know no examples of alternatives one and three have been reported in the literature to date. This does not mean that they would not occur. It may be that the time intervals between successive observations were not optimal for such events to be documented. If we want to emphasize that we are dealing with binary trees containing occasional aggregates of bifurcations (e.g. Fig. 12.3B or C), we speak of ‘essentially’ binary trees as opposed to ‘strictly’ binary trees. However, if we assume that the

![Fig. 12.3 Three alternative schemes of the formation of a trifurcation. (A) A real multifurcation is formed if the growth cone splits into three parts. (B) An aggregate originates if very close to an already existing bifurcation point a new growth cone protrudes. (C) An aggregate might also be formed when a growth cone divides into two parts, one of which again divides close to the first bifurcation point. The circle indicates that the two points are too close to be observable separately.](image-url)
observed multifurcations are real (e.g. Fig. 12.3A), it may be better to speak of mixed trees. As indicated in Fig. 12.3 the appearance of the resulting tree does not provide any clue as to how the multifurcations were formed. Apart from branching and extension of neurites, it is also quite common that segments or even entire branches are retracted (cf. Speidel 1932, 1942, 1964; Bray 1973; Bray and Bunge 1973). For instance, the outgrowth and withdrawal of goldfish retinotectal axon arbors continues throughout their lifetime. The reason for this process is that the retina and tectum grow in different ways and the axons in the tectum have to shift to maintain homotopic connections (cf. Easter and Stuemer 1984; Stuemer 1984). A related observation made by Speidel concerns the apparent shortening of an intermediate segment causing branching points to fuse (Fig. 5 in Speidel 1932). This is interesting with respect to the above-mentioned occurrence of multifurcations. The reverse process, namely a real multifurcation splitting up by interstitial segment growth, has been examined as a possible model, but was dismissed subsequently because its predictions did not match with data from real trees (cf. Berry and Bradley 1976). However, we cannot exclude the possibility that this latter process occurs occasionally. It may be important to keep in mind that the membrane and cytoskeleton are not as rigid as the appearance due to Golgi stain suggests (cf. Stryer 1988).

12.4 Measures and labels of topological properties

If we want to describe certain features of the shape of a neuronal tree structure that are deemed to be important for our research, it is necessary to do so as concisely as possible. This is important, if we need to analyse our observations, and if we wish to present our data to others. For such purposes we define labels and summarizing quantities (measures) for the topological properties. Before we discuss a number of summarizing variables a list of criteria, which in our view are important for topological labels or measures, will be given.

12.4.1 Criteria for a topological label or measure

*It should be simple*  This pertains to both its determination and its interpretation.

*It should be discriminative*  This means that small changes in the property, summarized by the label or measure, can be detected.

*It should incorporate as much information as possible*  One may sometimes prefer to use a measure or label that incorporates less information if it is easier to determine and only gross effects are of interest.

*It should be independent of degree*  This may seem an odd criterion, since degree (cf. Section 12.4.2) is a measure itself and all other measures are to some extent dependent on the degree. But the differences in degree between two groups of trees may dominate the differences found in the measure that we are actually interested in.
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For instance, if we want to compare dendritic trees at different stages of development, it is obvious that between certain stages the average number of segments per tree will change. The dependence on degree should be accounted for in the analysis.

*It should be meaningful if multifurcations are present* The value of a measure or label should not become indeterminate if a multifurcation is encountered. Otherwise loss of information is unavoidable.

*It should enable subsequent statistical testing* Clearly, the ultimate aim of a measure is to use it in comparisons or to decide whether or not our ideas about the shape are correct. It appears that it is not always easy to find appropriate statistical tests for topological measures. It is very important to realize that all topological variables are discrete.

### 12.4.2 Degree (n)

This is one of the simplest measures of tree topology. As mentioned before (cf. Fig. 12.2C) degree designates the number of terminal segments or tips (cf. Harding 1971). It contains only very limited information, but that may be completely satisfactory in some situations. Degree is insensitive to multifurcations (cf. Fig. 12.4). It can be used for each tree or for each neurone and alternatively as mean degree per neurone for each animal.

### 12.4.3 Number of branching points (b)

Most of the properties are similar to those of degree. In strictly binary trees there is a one-to-one relation between degree and number of branching points (see Section 12.2). This measure is highly sensitive to the occurrence of multifurcations (cf. Fig. 12.4). For trees containing many multifurcations it might sometimes be useful to score both degree and number of branching points. Then a bivariate analysis could be attempted or we might combine both variables (e.g. in a ratio b/n).

![Diagram of trees](image)

**Fig. 12.4** Illustration of the effect of a multifurcation on degree (n) and number of branching points (b). (A) the number of branching points is n - 1. (B) The occurrence of one trifurcation reduces the number of branching points with 1, whereas the degree is unaffected.
12.4.4 Topological type

The topological type is a standard representation of binary trees that are topologically identical. According to rules given by Harding (1971) and van Pelt and Verwer (1983) binary topological types can be classified and to each type a unique number (label) may be assigned. This is illustrated in Fig. 12.5 for the first 14 trees. The logic of the classification can easily be discerned from Fig. 12.6. For trees of the same degree the first topological type is intuitively the most asymmetric (unbalanced) and as the type number increases the types become more symmetric (balanced). In principle the topological type number is the ideal summary of the topological properties of a binary tree. For practical purposes it is totally unsuitable. The number of possible tree types increases very rapidly with increasing degree, making this label unmanageable for statistical testing (cf. van Pelt and Verwer 1984a). The presence of occasional multifurcations makes the number of possible types grow even more dramatically.

12.4.5 Centrifugal order ($\gamma$)

The centrifugal order of an element (e.g. segment, tip, or branching point) is the number of segments between the root and that element (cf. Fig. 12.2F). Other ordering systems that have been proposed are reviewed by Uylings et al. (1975) and MacDonald (1983), but centrifugal ordering seems to be the most useful for neuronal trees. Its properties have been studied quite extensively (cf. van Pelt et al. 1989, 1990). If the order distribution of segments is used one must be aware that it may contain a lot of redundant information and the number of independent observations is less than the total number of segments (cf. Verwer and van Pelt 1986). To get around this problem

![Diagram of binary topological tree types]

**Fig. 12.5** The first 14 binary topological tree types. The degree of each type is indicated. For analysis in terms of growth models the first three types are irrelevant, because in growth models the relative frequencies of types having the same degree are important (see also Fig. 12.15 and Table 12.2). Reproduced from Verwer and van Pelt (1986) with permission.
Fig. 12.6 Schematic drawings of all topological types for binary trees up to degree 8; *i* is the relative number for a given degree *n*; *r, s* represent the partition of terminal segments over the two first-order subtrees. Reproduced from van Pelt and Verwer (1983) with permission.

the mean segment order per tree could be determined (Fig. 12.7A), but this measure still depends in a complicated manner on the degree (see Sections 12.5 and 12.6). Order is affected by the presence of multifurcations (Fig. 12.7B).

12.4.6 Height (*h*)

Height is the length of the longest path in a tree from the root to some terminal tip in terms of segments (cf. Fig. 12.8). It is equivalent to the highest centrifugal order of any terminal tip (cf. Harding 1971; Tucker 1980). In geography this measure is called diameter (see also Uylings et al. 1989a), but it seems advisable to avoid confusion with the metric diameter of segments. Height contains more information
Fig. 12.7 The effect of a multifurcation on the mean centrifugal order \( E(\gamma) \) of segments. (A) A binary tree. (B) A tree with the same degree and one trifurcation.

Fig. 12.8 (A) The height of a tree is the length of the longest path in terms of segments from the root to some terminal tip. (B) The effect of a trifurcation in a tree having the same degree as in (A). It may be noted that the height is unaffected if multifurcations occur in other paths than the longest.

than degree but still a large amount of information is ignored. It is a simple measure and depends on degree. Problems may arise if multifurcations are present (cf. Fig. 12.8B).

12.4.7 Partitions

A partition is not a real measure or label, but a distribution. The term is not specific for trees (cf. Tucker 1980; Biggs 1985; Grimaldi 1985). We use it to indicate how the terminal segments are distributed over the subtrees of a branching point (cf. Figs 12.2D, E, 12.4 and 12.6). But we might equally well use it for the distribution of branching points over the subtrees (Verwer and van Pelt 1987) and many other features (see also Section 12.7). The idea of a partition is best understood if we imagine a number of
coins that we want to divide between some piles, which are regarded as indistinguishable (cf. Tucker 1980). If we have \( n \) coins and \( m \) piles the partition \((r_1,\ldots,r_m)\) denotes one possible distribution of the coins, with \( r_1 + r_2 + \ldots + r_m = n \). In Table 12.1 the possible partitions for a few values of \( n \) and \( m \) have been listed. How we take a dendrite apart into partitions is shown in Fig. 12.9. Note that we have indicated the partitions by \( n \), \( r_i \) instead of \((r_1, r_2)\), because it is easier for detecting mistakes in scored trees. The distribution of observed partitions in a group of trees depends on the degree, but by using a certain lumping scheme this effect can be eliminated (Verwer and van Pelt 1986; Verwer et al. 1987). The partition distribution provides a drastic data reduction with a minimal loss of information as compared with topological type (van Pelt and Verwer 1984a; Verwer and van Pelt 1983).

Table 12.1 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 is divided among these subtrees. Reproduced from Verwer and van Pelt (1990) with permission.

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Fig. 12.9 The proportional sum of absolute deviations (PSAD) of terminal segments of (sub)trees is determined using partitions. (A) A schematic copy of an aspiny neurone from the human striatum shown in Fig. 15.2 of Graveland et al. (1985). (B) Display of dendrites in terms of partitions, indicated as \( n_r \) instead of (\( r_n \)). (C) The corresponding display of PSAD values and the mean PSAD values (PSAD) per dendrite. (D) PSAD value of the soma and the mean PSAD of the entire neurone, in which equal weight is given to the PSAD value of the soma and those of each partition in the trees. Reproduced from Verwer and van Pelt (1986) with permission.
12.4.8 Proportional sum of absolute deviations (PSAD)

This measure is directly related to partitions (Fig. 12.9). It is not specific for trees and we use it to indicate how much the distribution of terminal segments over the subtrees of a branching point deviates from a uniform distribution (cf. Fig. 12.10). Actually, it is a ratio of two dispersion measures (cf. Verwer and van Pelt 1986).

\[
\text{PSAD} = \frac{m}{2(m-1)(n-m)} \sum_{i=1}^{m} \left| r_i - \frac{n}{m} \right|
\]

where \(2(m-1)(n-m)/m\) is the sum of absolute deviations (SAD) of the partition \((1, \ldots, 1, n-m+1)\) and the rest of the right-hand side is the SAD of the partition \((r_1, \ldots, r_m)\), for which we wish to determine the PSAD. Why did we not use a ratio of variances instead of PSAD? Firstly, PSAD is a generalization of a measure for binary partitions that was termed asymmetry (van Pelt and Verwer 1986), and secondly it is much simpler than a ratio of variances. PSAD might also be used for other features (i.e. branching points etc.) if desired (Verwer and van Pelt 1987). For each partition the corresponding PSAD might be determined and the mean value be used as an indication of asymmetry for a whole tree or an entire neurone (cf. Fig. 12.9). PSAD is only mildly

![Diagram](image_url)

**Fig. 12.10** Explanation of the proportional sum of absolute deviations (PSAD). PSAD indicates the deviation from a uniform distribution of terminal segments over the subtrees of a branching point (in this case a 5-furcation with 30 terminal segments). The left-hand part shows that PSAD = 0 for a uniform distribution. The most extreme distribution of terminal segments, corresponding to PSAD=1, is shown in the right-hand part. The middle part shows some intermediate distribution.
dependent on degree (van Pelt et al. 1990; see also Section 12.6) and can be used in
the presence of multifurcations (Verwer and van Pelt 1986; Sections 12.5 and 12.6).
The information content of PSAD is between that of the partition distribution and the
distribution of centrifugal order.

12.4.9 Vertex ratio (VR)

This measure was introduced by Sadler and Berry (1983) and defined in terms of binary
vertices (see Chapter 13 in this book). It might also be expressed as follows:

\[
VR = \frac{T(2)}{n - 2T(2)},
\]

where \( T(2) \) is the number of subtrees of degree 2 and \( n \) is the degree of the entire tree
(cf. Fig. 12.11). Statistical testing requires caution if VR is determined for each tree
separately, because any tree for which \( n = 2T(2) \) will have an infinite VR. Therefore,
for groups of trees of the same degree (\( n \)) the mean vertex ratio \( E(\text{VR}) \) is defined as

\[
E(\text{VR}) = \frac{E[T(2)]}{n - 2E[T(2)]},
\]

where \( E[T(2)] \) is the mean number of second-degree subtrees per tree. In general this
measure depends on the degree, although, in particular situations it is independent (see
Section 12.6). For trees of a particular degree the vertex ratio is determined by
counting the number of subtrees of degree 2. Consequently, if aggregates occur in which
more than two terminal segments are involved, the number of subtrees of degree 2
cannot be established (cf. Verwer and van Pelt 1985). Thus, the vertex ratio is indeter-

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**Fig. 12.11** The vertex ratio (VR) is determined by the degree of a tree and the number \((T(2))\)
of subtrees with two terminal segments (indicated by the heavy lines). (A) A binary tree with
two second-degree subtrees. (B) A trifurcation that does not interfere with the determination of
\(T(2)\). (C) If this trifurcation corresponds to an aggregate it renders \(T(2)\) indeterminable. The
original tree then might have been either A or D. (D) A binary tree with one second-degree subtree.
mininate for trees containing such aggregates (cf. Fig. 12.11). For all other aggregates
the vertex ratio is insensitive. It should be noted that real multifurcations do not interfere
with the determination of \( T(2) \), that is, the vertex ratio does not use the information
contained by real multifurcations. The amount of information incorporated by the vertex
ratio is rather low as measured by its value domain (Uylings et al. 1989a; van Pelt et
al. 1990).

12.4.10 Terminal/link ratio (T/L)

The terminal/link ratio has been defined for each centrifugal order \( (\gamma) \) as the number
of terminal tips at the next order \( (n_{\gamma}(\gamma + 1)) \) divided by twice the number of bifurca-
tions \( (\text{bif}(\gamma)) \) plus three times the number of trifurcations \( (\text{trif}(\gamma)) \):

\[
T/L(\gamma) = \frac{n_{\gamma}(\gamma + 1)}{2\text{bif}(\gamma) + 3\text{trif}(\gamma)}
\]  

(cf. Sadler and Berry 1988). If multifurcations other than trifurcations are observed
the definition of the T/L ratio must be extended accordingly. Since \( n_{\gamma}(\gamma + 1) \) equals
\( n(\gamma) \) (cf. Fig. 12.2F), it is easy to verify that the T/L ratio can be reformulated as the
number of terminal segments \( (n(\gamma)) \) divided by the total number of segments \( (s(\gamma)) \)
at each order

\[
T/L(\gamma) = \frac{n(\gamma)}{s(\gamma)}
\]  

(cf. Fig. 12.12). A similar measure (i.e. \( 1 - T/L(\gamma) \)) was used by Smit et al. (1972). The
properties of the T/L ratio are expected to be comparable with those of the centrifugal
order, but this needs to be studied further. Practical applications of the T/L ratio are
given in detail by Berry in Chapter 13.

![Diagram](image)

**Fig. 12.12** Illustration of the determination of the terminal–link ratio (T/L) according to
equation 6. (A) A binary tree in which e.g. T/L(3)=3/4, because we have three terminal
segments and one intermediate segment at centrifugal order 3. Note that the differences
between order of segments and of branching points may easily cause confusion about the T/L
values (cf. Fig. 12.2F). (B) The effect of a trifurcation. To obtain a summary value per tree the
mean T/L ratio \( (E(T/L)) \) might be used.
12.5 Comparison of groups

In many investigations the aim is to compare tree data from two or more groups that differ in experimental treatment or pathological diagnosis. Several considerations may lead to the choice of a particular measure. One aspect that must be considered is the level at which we wish to compare the observations. If we are willing to assume that each branching point constitutes an independent decision of a tree, then we might use the branching points as independent observations. In this way we will generally have a large number of observations. Very suitable for such analysis would be the PSAD and partitions. Less suitable, because of inherent dependence, are the distributions of the centrifugal order and T/L ratio. Alternatively we might want a summary for each tree and use the trees as independent observations. Partitions cannot be used in such an approach, because there is no way to summarize them into one value for each tree. However, the mean PSAD, height, mean centrifugal order, vertex ratio, or mean T/L ratio could be used. For certain trees the vertex ratio yields infinite values. For large trees this property may not be serious, because in practice such particular trees have a rather low probability of being observed. One could also use the mean vertex ratio (see above) and apply a jackknife procedure producing as many pseudo-values as there are observed trees by deleting one tree at a time (cf. Wonnacott and Wonnacott 1985). Otherwise, the inverse vertex ratio as suggested by Horsfield and Woldenberg (1986) may be used. A summary for a neurone possessing more than one dendrite can be found using the overall values of mean PSAD, mean centrifugal order, vertex ratio, mean T/L ratio, or mean height. If we wish to compare the topological properties of neuronal trees for groups of animals (c.q. patients) we could sample only one exemplar of a neuronal tree per animal or else take the mean value of several trees per animal.

12.5.1 Effect of degree

If all observed trees are strictly binary any measure or label can be used. However, all are in some way dependent on degree, which means either that only trees of the same degree are compared, or that regression must be applied. In the first instance as many parallel statistical tests must be performed as there are different degrees. The tests should be combined afterwards (cf. van Pelt and Verwer 1984a). The dependence on degree is in general non-linear, and this is most pronounced for smaller degrees. As a second solution a non-linear regression might be attempted for each group of data followed by a comparison of the regression coefficients, which is not a trivial task. An alternative is to use a parametric regression approach. For instance, the parameters of any growth model could be used to construct the best fitting regression curve (cf. van Pelt et al. 1989; Section 12.6). Subsequently, the estimated parameters of the best fitting curves estimated via a jackknife approach (cf. Wonnacott and Wonnacott 1985) could be used to compare between groups. It has been suggested that we minimize the effect of degree by standardizing the measures concerned (cf. Uylings et al. 1989a). The standardized measure $M_s$ is defined as

$$M_s = \frac{M_{\text{obs}}(n) - M_{\text{min}}(n)}{M_{\text{max}}(n) - M_{\text{min}}(n)}$$

(7)
such that its value is in the interval [0, 1]. Here, $M_{\text{obs}}(n)$ is the observed value of the measure, $M_{\text{min}}(n)$ is the smallest possible value of the measure for degree $(n)$ and $M_{\text{max}}(n)$ is the largest possible value. Note that PSAD is by definition already standardized. The effect of degree on partitions can be minimized by lumping the partitions and using conditional probabilities (cf. Verwer and van Pelt 1983, 1986; Verwer et al. 1987). Both regression and standardization need to be studied further to permit advice about a preferred procedure. Certainly, standardization is easier to apply.

12.5.2 Comparison and multifurcations

If multifurcations are observed we need to make assumptions. Real multifurcations (i.e. those inherent in the unknown growth process) will not pose any problem for partitions, PSAD, T/L ratio, or vertex ratio and probably likewise for the mean centrifugal order. However, if the multifurcations are actually aggregates of bifurcations we run into problems. For example, aggregates imply that some segments are unobservable, thus invalidating both mean T/L ratio and mean centrifugal order. Similarly, the PSAD value of an observed aggregate may be quite different from that corresponding to the unidentifiable sequence of bifurcations (cf. Verwer and van Pelt 1986). It is at present unclear whether partitions of aggregates are allowed. Thus, if we cannot discriminate between a real multifurcation and some indeterminable sequence of bifurcations, further comparative analysis depends on whether we are prepared to assume either possibility. If we assume that the observed multifurcations are aggregates we could use the PSAD for the obvious bifurcations and ignore any aggregate. We have to resign ourselves to being unable to recover the missing information.

12.6 Growth models

In Section 12.3 we discussed some aspects of the dynamics of neuronal trees. The way that the trees have grown undoubtedly has implications for the variation in topological types that will be observed. Although it would be preposterous to think that we could mimic the growth process by modelling, growth models may help us explain the observations and thus contribute to our understanding of the process. We have no idea why a growth cone divides (see also Section 12.3). Bray (1973) noted that ‘this process is apparently random’. For this reason the growth models have a probabilistic nature. Growth models provide a set of rules giving a simple description of how the growth process might occur. We will restrict ourselves here to the branching process (topological aspects) only and omit the elongation and shortening of segments (metrical aspects). As we have seen in Section 12.3 these metrical actions may have an impact on the topological appearance of trees. We will also discuss the implications for growth models and analysis. The approaches to modelling the branching process that have been studied so far can be categorized into sequential and synchronous growth (cf. Horsfield et al. 1987). The difference between these two categories is that in sequential growth each branching event consists of the formation of one new branching point, whereas in synchronous growth several new branching points may arise simultaneously during a single branching event (Fig. 12.13). Yet another
Fig. 12.13 Illustration of the difference between sequential (A) and synchronous (B) growth. (A) In sequential growth each branching event involves the division or protrusion of one growth cone (asterisk). (B) In synchronous growth several growth cones (asterisks) may divide or protrude during a single branching event.

approach, which is beyond the scope of this review, was proposed by Klémann (1987). This involves the determination of splitting probabilities based on the T/L ratio for each order, which can be used to calculate the probability of any possible tree. A more restricted version of this model was previously proposed by ten Hoopen and Reuver (1970).

12.6.1 Sequential binary growth models

Two particular cases of the so-called sequential binary growth models (i.e. terminal and segmental growth) were originally imported into neurobiology from the field of geography (see Berry et al. 1975; Hollingworth and Berry 1975). Initially, the expected frequencies of trees were determined using computer simulations (Smit et al. 1972; Berry et al. 1975). Starting from the work of Harding (1971), who derived the probabilities of occurrence of topological types of phylogenetic trees under terminal growth, we could derive a large range of sequential growth models (van Pelt and Verwer 1983, 1985, 1986). These models include both terminal and segmental growth as particular cases. The binary growth models distinguish between terminal and intermediate segments by means of the parameter Q and take the effect of the order into account via parameter S.

Assumptions are:

1 At each branching event only one segment branches.
2 At each branching event one branching point is formed and the existing segment is replaced either by one intermediate and two terminal segments or by two intermediate and one terminal segment.
3 Each branching event is independent of all previous events that took place in the same tree.
4 The probability of a branching event occurring at an intermediate segment is equal for all intermediate segments of the same order \( p_i(\gamma) \).
5 The probability of a branching event occurring at a terminal segment is equal for all terminal segments of the same order \( p_t(\gamma) \).

Figure 12.14 illustrates the effect of different values of the parameters Q and S in the sequential growth models. The width of the segments is an indicator for the
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Fig. 12.14 The effect of the growth parameters $Q$ and $S$ on the branching probabilities of intermediate and terminal segments at different centrifugal orders. The square root of the relative branching probability of each segment is proportional to its diameter in this diagram.

relative probability of forming a new branch. It can be seen that if the parameter $Q = 0$ only terminal segments are allowed to branch (i.e. $p_i = 0$). As the value of $Q$ increases the probability of intermediate segments branching relative to that of terminal segments increases too. And if $Q = 1$ only intermediate segments can branch (i.e. $p_i = 0$). The value of the parameter $S$ ranges from $-\infty$ to $+\infty$. Negative values of $S$ favour branching of more distal segments, whereas positive values of $S$ favour branching of more proximal segments. If $S = 0$ then there is no effect of centrifugal order. The influence of the branching probabilities $p_i(\gamma)$ and $p_i(\gamma)$ on the relative frequency of topological types is illustrated in Fig. 12.15. For a number of values of $Q$ and $S$ the corresponding probabilities and type frequencies are presented in Table 12.2.

12.6.1.1 Analysis using sequential growth models

Several measures and labels have been used in the past to test neuronal trees in terms of sequential binary growth models. To be useful for analysis of growth models the values of measures or labels must be functions of the growth parameters. Further, we can take either of two approaches. One approach consists of assuming a hypothetical mode of growth and testing whether the data agree with the hypothesis (Verwer and van Pelt 1983). The other involves estimating the parameters of the most likely growth model using minimum chi-square or maximum likelihood estimation (van Pelt and Verwer 1986; Verwer et al. 1987). The $Q/S$ models do not predict when a tree stops branching. Therefore, neither the degree nor the number of branching points can be used for this kind of analysis. Probabilities of occurrence of partitions can easily be
Fig. 12.15 Explanation of the effect of the branching probability of each segment on the relative frequency of topological types of the same degree. In a binary tree of degree 3 (top) there are five possible branching events, each giving rise to either of the two possible tree types of degree 4 (bottom). In the middle part of the figure the branching probability of the segment indicated by the arrow is given. For instance, \( p_t(0) \) denotes the branching probability of an intermediate segment of order zero.

Table 12.2 The relative frequencies of the two topological tree types of degree 4 for a few growth models (cf. Fig. 12.15). Comparison with Fig. 12.14 may be helpful to interpret the resulting branching probabilities.

<table>
<thead>
<tr>
<th>Order</th>
<th>Branching probability</th>
<th>( (0,-1) )</th>
<th>( (0,1) )</th>
<th>( (0.5,0) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>( p_t(0) )</td>
<td>0</td>
<td>0</td>
<td>1/5</td>
</tr>
<tr>
<td>1</td>
<td>( p_t(1) )</td>
<td>0</td>
<td>0</td>
<td>1/5</td>
</tr>
<tr>
<td>1</td>
<td>( p_t(1) )</td>
<td>1/5</td>
<td>1/2</td>
<td>1/5</td>
</tr>
<tr>
<td>2</td>
<td>( p_t(2) )</td>
<td>2/5</td>
<td>1/4</td>
<td>1/5</td>
</tr>
<tr>
<td>2</td>
<td>( p_t(2) )</td>
<td>2/5</td>
<td>1/4</td>
<td>1/5</td>
</tr>
</tbody>
</table>

Relative frequency of

- tree type 1 (asymmetric) 4/5 1/2 4/5
- tree type 2 (symmetric) 1/5 1/2 1/5
expressed in terms of the parameters $Q$ and $S$ (van Pelt and Verwer 1983, 1985, 1986). In Table 12.3 the maximum likelihood estimates of $Q$ and $S$ of retinotectal axons from three areas of the goldfish tectum are given. The partitions were scored from camera lucida drawings of Stuermer (1984), thereby ignoring all multifurcations (see also Verwer et al. 1987; Verwer and van Pelt 1990). The results may be interpreted as follows (see also Fig. 12.14). During the growth of peripheral axons the terminal segments have a slightly higher probability of branching than intermediate segments and there is a tendency to favour more distal segments. Of course, one should be careful with interpreting these data, since the influence of withdrawal of branches is unknown (see also Sections 12.3 and 12.6.3). Using mean centrifugal order the same conclusions were obtained (cf. van Pelt et al. 1989). We have used mean order to analyse the dendrites of pyramidal and non-pyramidal cells from a study of McConnell and Uylings (cf. Uylings et al. 1983; van Pelt et al. 1990). From the results shown in Fig. 12.16 it can be seen that mean order depends in a non-linear way on degree and that the data can be fitted quite well using this measure. The fact that the reduced chi-square statistic is either 1 or very close to 1 indicates that the models defined by the maximum likelihood estimates of $Q$ and $S$ explain all observed topological variability. It appears that both pyramidal and non-pyramidal cells form new segments at terminal segments with a small preference for the more proximal ones. The vertex ratio has been used to compare Purkinje cells with terminal and segmental growth (cf. Sadler and Berry 1983). The expected values are

$$E_t(\text{VR}) = 1$$  \hspace{1cm} \text{terminal growth} \hspace{1cm} (Q = 0) \hspace{1cm} (8)$$

$$E_s(\text{VR}) = \frac{n-1}{2(n-2)}$$  \hspace{1cm} \text{segmental growth} \hspace{1cm} (Q = 0.5) \hspace{1cm} (9)$$

(cf. Verwer and van Pelt 1985) but since the distribution of VR under the growth hypotheses is unknown, it has been proposed that we use the Monte Carlo test to find

<table>
<thead>
<tr>
<th>Area</th>
<th>Maximum likelihood estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Q$</td>
</tr>
<tr>
<td>Central tectum</td>
<td>0.00</td>
</tr>
<tr>
<td>Intermediate tectum</td>
<td>0.14</td>
</tr>
<tr>
<td>Peripheral tectum</td>
<td>0.34</td>
</tr>
</tbody>
</table>
Fig. 12.16 (c) Averaged mean-order values of a set of 443 pyramidal cell basal dendrites having different degrees. Error bars indicate the standard deviation. Degree 12 and 14 were only represented by one tree. Some model curves for different values of $Q$ and $S$ are included for comparison. Compact (COMP.) refers to populations consisting of the most symmetrical (balanced) trees and THIN refers to populations of the most asymmetric (unbalanced) trees. (a) The reduced chi-square statistic for $Q=0$ and several values of $S$ attains a minimum value of 1 for $S=0.59$, indicating that all topological variability in the observations can be explained by a growth model with $(Q=0, S=0.59)$. (d and b) Similar presentations of the results for 487 multipolar non-pyramidal cell dendrites. Degrees 11, 12, 13 and 14 were only represented by one tree. Reproduced from van Pelt et al. (1989) with permission.

significance levels for observed data (Verwer et al. 1985). It may be noted that the simulations inherent in the Monte Carlo test are based on the partition probabilities. Apart from terminal growth ($E_i(VR) = 1$) the vertex ratio depends in a non-linear way on the degree for other growth models. However, for very large trees ($n \rightarrow \infty$) Horsfield et al. (1987) showed that

$$E(VR) = 1 - Q.$$ (10)
This means that if we have very large strictly binary trees, an estimate of $Q$ can be obtained directly from $E(VR)$. The other measures (i.e. height, T/L ratio and PSAD) have not been well studied with respect to binary growth models, but PSAD seems to have some attractive properties. Simulation of binary trees of different degree shows that the mean PSAD per tree is only very slightly dependent on degree (Fig. 12.17; van Pelt et al. 1992). The variability quickly decreases with increasing degree. If we could assume that observed multifurcations actually are aggregates of bifurcations (see also Section 12.3), we could apply a modified binary growth model analysis using partitions (Verwer and van Pelt 1990) or the PSAD.

![Diagram](image)

**Fig. 12.17** Mean PSAD values of 497 trees having different degrees between 0 and 500 were generated by computer simulations using a growth model defined by $(Q=0, S=0)$. The total mean PSAD value (0.591) is affected very little by the degree. Only the variability is higher for smaller degrees.

### 12.6.2 Synchronous binary growth models

The idea of synchronous growth of binary trees has been proposed (cf. Horsfield et al. 1987) as an alternative to sequential growth, with the intention of using it to explain the mode of growth of bronchial trees. In this model growth is considered as a series of branching events (‘time steps’) during which 0, 1 or even more segments may bifurcate. The model is defined by branching probabilities for intermediate and terminal segments which are fixed during the entire growth process. This is in contrast to the sequential growth model where the sum of the branching probabilities is normalized to 1 for each branching event. By its definition synchronous growth needs a third parameter to account for the number of branching events that have occurred between the origin of the trees and the moment of observation. Without this extra parameter the observations are untestable. An illustration of two possible steps in a synchronous growth process is given in Fig. 12.13B. Synchronous growth certainly has some attractive features, but it needs to be studied more extensively before it can be applied to analyse real data.
12.6.3 Some general remarks concerning models

There are some aspects of the dynamics of trees that have not been considered in the previous sections. One is that the models discussed do not incorporate actions such as withdrawal of branches. For growth models defined by the parameter \( Q \) we have investigated the effect of random loss of branches on the topological properties, which was found to be negligible (van Pelt and Verwer 1984b). Incorporation of withdrawal of branches in the growth models would imply extra parameters that should be evaluated with the observed tree data. This is not very attractive, firstly because it is not at all obvious how these parameters should be defined and secondly because the parameters \( Q \) and \( S \) suffice to explain the observed topological variability of, for instance, neocortical pyramidal and non-pyramidal cells (Fig. 12.16; van Pelt et al. 1989). Consequently, we run a fair chance of ending up with an over-parametrized model with little explanatory value.

If the occasionally observed multifurcations are real, that is, they are inherent in the growth process, it is impossible to analyse the trees in terms of binary growth models. In fact, it is necessary to define the probability that a branching event involves a multifurcation. However, it appeared to be impossible to derive analytical formulae for the resulting partition probabilities (Verwer and van Pelt, in preparation). These probabilities must be evaluated using computer simulations. Sadler and Berry (1988) simulated trees containing inherent trifurcations in conjunction with the T/L ratio. It will be clear that the resulting growth models (mixed growth) are definitively different from the binary growth models, which could also be simulated if one wished to do so. To generate essentially binary trees with occasional aggregates the probability of aggregation could be estimated from the data. These two latter approaches (i.e. generating strictly and essentially binary trees) are unnecessary since analytical procedures are easily applicable (cf. van Pelt and Verwer 1985, 1986; Verwer and van Pelt 1990). Before we start an analysis we must first make assumptions about which ensemble of growth models (i.e. strictly binary, essentially binary with aggregates, or mixed) would be appropriate.

12.7 Concluding remarks

We have reviewed some descriptors of topological properties of trees that might be used when we wish to analyse neuronal branching patterns. Criteria that could be used to evaluate these descriptors have been given. None of the descriptors has yet been studied well enough to permit a definitive judgement. Especially, their performance in statistical tests needs further careful investigation. Based on our current knowledge we have a preference for the use of the PSAD. It must be emphasized that the observed topological variability is a consequence of the way that the trees have grown in the broadest sense. It appeared that in order to analyse neuronal trees (either in comparisons or in terms of growth models) we have to make assumptions about the unknown dynamical processes that underlie the formation of the trees (e.g. origin of multifurcations, synchronous branching, etc.). For the ultimate interpretation it may also be necessary to incorporate eventual knowledge about withdrawal of branches.
Further, we would like to mention some topics that are interesting with respect to the methodology presented in this review. We have used partitions to describe the division of terminal segments over the subtrees of branching points. In line with this approach partitions of spines or synapses could be defined. For this purpose it may be necessary to reconstruct cells from ultra-thin sections (cf. White 1979; Sterling et al. 1986). In relation to the distribution of synapses over the dendritic trees of neurones it is possible to identify dendritic subunits in retinal ganglion cells which are electrically homogeneous (cf. Koch et al. 1982). In Fig. 12.18A a schematic impression of such a cell is shown. The subunits could be considered as leaves (cf. Fig. 12.18B) or terminal tips of the cell, whose topological and electrical properties could be analysed together. If the electrical contribution of each leaf is the same we might use them as unlabelled tips, otherwise we might assign labels to them if we wish (cf. Fig. 12.18B). In Purkinje cells it is possible to distinguish spiny branchlets from the smooth primary dendrites (Hollingworth and Berry 1975; Shelton 1985). The topo-

![Fig. 12.18](image1.png)  
**Fig. 12.18** (A) A schematic drawing of a neurone having dendritic subunits with homogeneous electrophysiological properties. (B) Each subunit might be considered as a terminal tip (labelled or unlabelled) for a combined morphological/electrophysiological interpretation or analysis.

![Fig. 12.19](image2.png)  
**Fig. 12.19** (A) A schematic drawing of a Purkinje cell with smooth primary dendrites (black) and spiny branchlets. (B) The topological properties of the smooth dendrite might be investigated by considering each spiny branchlet as a terminal tip or leaf. The leaves could be labelled if we wish. Likewise, we might want to analyse the topological properties of the (labelled or unlabelled) leaves (spiny branchlets) separately.
logical properties of these two components are significantly different (our unpublished analysis of camera lucida drawings from papers of Berry et al.). Shelton (1985) has used the anatomical distinction between the smooth dendrite and the spiny branchlets to model the electrical properties of the Purkinje cell dendrite. In Fig. 12.19 it is illustrated how labelled leaves can be used. For other studies it might be helpful to use labelled or unlabelled leaves to identify parts of dendrites that are located in areas of particular interest.

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References


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