

NSM 00592

Application of growth models to the topology of neuronal branching patterns

J. van Pelt, R.W.H. Verwer and H.B.M. Uylings

Netherlands Institute for Brain Research, Amsterdam (The Netherlands)

(Received 24 February 1986)

(Revised 10 April 1986)

(Accepted 19 April 1986)

Key words: Dendritic trees — Branching patterns — Topology — Growth model — Subtree partition analysis

The variation in topological structure of branching patterns may contain essential information with respect to the way these branching patterns have grown. For the understanding of how growth modes finally result in a particular variety in topological patterns, model studies may provide indispensable tools. These studies imply the mathematical formulation of growth models and the development of statistical procedures to compare model predictions with observed data. Recent literature shows two main approaches in these model studies, viz. subtree partition analysis (SPA) and vertex analysis. This paper will briefly review the current status with respect to SPA and will apply the model approach to sets of dendritic trees taken from pyramidal, multipolar non-pyramidal and from Purkinje cells. The results show that the topological properties of many dendrites are not in agreement with the hypothesis of random terminal growth and that substantial branching of intermediate segments and/or branching dependent of the position of segments in the tree (topological distance from the cell body) must be assumed. Only two parameters are required to incorporate these assumptions in the model. In all cases up to now it is possible to find parameter values such that the model predictions of topological properties are in agreement with the observations.

Introduction

The shape of branching patterns is the result of a growth process that is usually governed by many interacting factors. If it is assumed that all these interactions lead to regularity in the growth process, then it is interesting to know how this regularity is reflected in the properties of the final branching patterns. Alternatively, characteristic features of observed branching patterns may be indicative of regularity during growth. To approach this question one can perform developmental studies and look at changes in tree properties which may be indicative of the underlying growth

Correspondence: J. Van Pelt, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.

process and, possibly, may suggest certain rules about how the growth proceeds. If the results of one developmental stage only are available, then model studies are very fruitful, because growth can be simulated according to hypothesized rules and the model predictions may be compared with the observations. Also, if data are available from different developmental stages, such an approach is worthwhile because it may reveal changes in growth during development. The question of understanding branching patterns in terms of growth rules will, as a first approach, only concern the topology of the patterns. In other words, is it possible to understand the occurrences of different topological branching patterns and their frequencies by modelling the growth on the basis of hypothesized rules? This approach may lead to an interpretation of possible differences in structure either between branching patterns grown under different conditions, or between dendrites or axons of different neuronal types. The mathematical description of growth models, their properties and the development of the statistical procedures for testing tree properties have been described in several recent articles (Van Pelt and Verwer, 1983, 1984a, 1985, 1986; Verwer and Van Pelt, 1983, 1985, 1986; Verwer et al., 1986). For this reason, we will not go into detail on these aspects in this paper but only briefly review the procedure. We will focus especially on the application of these tools to analyze branching patterns described in two studies on the effect of starvation on dendritic growth of pyramidal and multipolar non-pyramidal cells (Uylings and McConnell, unpublished data) and Purkinje cells (McConnell and Berry, 1978).

Growth models

From a topological point of view branching patterns are formed by segments adopting a particular connectivity. A tree grows by increasing the number of segments, thereby also changing the topological pattern. We assume the trees to be binary and shall define a branching event in a topological way by the replacement of a segment by two consecutive segments and a branching point in between from which a third (terminal) segment emerges. The growth of a branching pattern will be described by a series of successive branching events which may occur on any segment in a tree, one at a time. Branching probabilities are assigned to all segments and they sum to one because only one segment branches per event. The branching events are thus the elementary steps in a stochastic process resulting in the assembly of trees.

The growth model is complete if the branching probabilities of all the segments are specified. In the following, we shall distinguish segments on the basis of their type (intermediate or terminal segments) and with respect to their topological distance to the root vertex, viz. the number of segments on the path from this segment to the root, called centrifugal order. If the growth model takes account of these distinctions in a well-defined way then the analysis of a set of trees will detect if the branching probabilities of segments are dependent on the segment type and/or segment position in the tree. For instance, a question frequently posed in

the literature is: does a tree branch randomly only at terminal segments or does it branch on all segments? The model distinguishes between intermediate and terminal segments by assigning separate branching probabilities P_i and P_t , respectively. Because the branching probabilities are normalized, it is sufficient to use one parameter Q , defined as $Q = P_i / (P_i + P_t)$ (cf. Van Pelt and Verwer, 1985). It is further assumed that the branching probability of a segment may depend on its centrifugal order. This dependency is incorporated in the model by an exponential relationship modulated by a second parameter called S such that $P_t = C \cdot 2^{-S\gamma}$ and $P_i = R \cdot P_t$, while $R = Q / (1 - Q)$, γ denotes the centrifugal order and C is a normalization constant (cf. Van Pelt and Verwer, 1986). A positive value of S implies that the branching probability of a segment decreases if it is further away from the root. A negative value has the opposite influence. The exponential form of S has been chosen to simplify the scheme of branching probabilities of all the segments. The orders of the segments in a (sub)tree are relative to the order of the root segment in the (sub)tree. If the order of the root segment would be changed by adding a constant, then the orders of all the segments change accordingly. Then the branching probabilities also change, but with the same multiplication factor, such that their mutual relationships remain unchanged. The Q - S model (Van Pelt and Verwer, 1986) is completely specified by the parameters Q and S . For $S = 0$, there is no order dependency, meaning that all segments of the same type (intermediate or terminal) have equal branching probabilities. Then, only the parameter Q is operative and we shall refer to this situation as the Q -model. For this model, Van Pelt and Verwer (1985) have derived mathematical expressions for the calculation of the probabilities of any tree type. We shall further refer to the S -model when $Q = 0$. Then, $P_i = 0$ and only terminal segments are allowed to branch dependent on their centrifugal order. Special cases occur in the Q -model ($S = 0$) for $Q = 0$ and $Q = 0.5$. For $Q = 0$ we have only branching of terminal segments and this model is called, in the literature, random terminal growth. For $Q = 0.5$, branching is possible at all segments with equal probability, and this model is called random segmental growth. Van Pelt and Verwer (1985) have shown that the Q -model is restricted in its prediction of tree properties. It is not able to predict trees with more mean symmetry in the degree (number of terminal segments) of subtrees at bifurcation points than is predicted by the random terminal growth model. The incorporation of an order dependency (Q - S model), however, has removed this shortcoming.

The Q - S model defines for each degree the probabilities of occurrence of all possible topological structures of trees. The topological structures that are distinguishable for 3-dimensional trees are called ambilateral types (Van Pelt and Verwer, 1983). Any property, derived from these topological structures, will be defined accordingly. For instance, an important property appears to be the 'partition', i.e. the way a tree partitions its terminal segments over the subtrees at a bifurcation point. Other characteristics are the order distribution of the segments or the distribution of their degrees. The prediction about each of these properties by the Q - S model may be compared with those derived from observed trees. For instance, the ambilateral type probabilities can be compared directly with the frequencies of observed tree types. This has a drawback, however, because the

number of different ambilateral types increases rapidly for larger degrees, making the use of its frequency distribution unmanageable. An efficient solution to this problem is the use of partition distributions, because: (1) the number of partitions per degree is much smaller than the number of ambilateral types; and (2) under the assumption of independency of the partitions within a tree there is no loss of information with respect to the growth model. The second argument means that it is sufficient to calculate the partition probabilities because the ambilateral type probabilities are easily calculated from these. The formulation of the Q - S model incorporates this assumption because the partition probabilities apply to any bifurcation point in the tree. The partition frequencies in a sample of observed trees are obtained by counting the number of terminal segments of the two subtrees at each bifurcation point in a tree, and combining the results for all the trees in the sample. Note that only partitions of at least degree 4 are relevant because for instance degree 3 always partitions as 3(1,2) while degree 4 can give 4(1,3) or 4(2,2) (Van Pelt and Verwer, 1983).

Parameter estimation

In applying these models, are we able to predict the observed frequency of topological patterns? For which values of the Q - S model parameters do the predictions correspond optimally to the observations? For this best-fitting model the one which predicts the sample with maximum likelihood will be taken (Wonnacott and Wonnacott, 1985). Let $L(P)$ denote the likelihood of the sample under predictions of the model defined by the parameter P . The maximum of $L(P)$ can be found by searching zero values of the first derivative of $L(P)$. However, for the Q model the first derivative is not described by a simple expression (Verwer et al., 1986) and for the Q - S model even the partition probabilities are only recurrently formulated (Van Pelt and Verwer, 1986). For these reasons, we obtained the maximum likelihood estimate (MLE) by calculating the likelihood for several values of the parameter(s) and looking for the maximum value. For the two-parameter model, the search proceeds as follows: take a start value in the parameter space at the centre of a 3×3 matrix with a certain spacing; calculate the likelihood for these 9 points and find the largest value; if the largest value is at a point, other than the centre, then make this point the centre of a new matrix and repeat the calculations; if the centre point produces the largest value then the matrix spacing may be reduced to find the maximum with a finer spatial resolution in the parameter space. The Q - S model, defined finally by the maximum likelihood estimate, will be denoted as the ML model. An impression of the shape of the likelihood landscape is obtained from a plot with several equi-likelihood contours in the Q - S parameter space. The contour plot indicates the position of the maximum in the likelihood function and the gradients indicate if it is the top of a sharp peak or of a broad hill.

Chi-square goodness-of-fit tests

The value of the likelihood itself is not a measure for the goodness-of-fit and thus the ML model may describe the data badly. The goodness-of-fit of any model (defined by Q and S) can be calculated by means of the χ^2 -test. In the calculation

of the level of significance, the degrees of freedom are corrected for the number of free parameters in the model. For example, the degrees of freedom in a test of a two-parameter ML model to a 6 class distribution is equal to $5 - 2 = 3$. For definitely, a priori stated hypotheses, like random terminal and segmental growth, there are no free parameters and no correction is applied. In the decomposition of trees, partitions may occur of any degree up to the number of terminal segments of the largest tree. For each degree a partition frequency distribution is constructed which has to be tested separately. The χ^2 -test is only applied to the distributions which meet the criteria of Cochran (1954), i.e. if the expected number of observations is at least one for each class, and at least 5 for at least 80% of the classes in the distribution. The partitions of the distributions, which do not meet this criteria, are subsequently reclassified in a composite distribution on the basis of the degree of the smallest subtree. Eventually, further lumping of the composite distribution is applied to increase the expected values per class (Verwer and Van Pelt, 1983, 1986). The significance levels obtained from the tests of all these distributions are finally combined by making use of the sum test and Fisher's test (Koziol and Perlman, 1978) as was described by Van Pelt and Verwer (1984a) resulting in an overall level of significance for the whole sample.

Summary of procedure

The topological analysis of branching patterns now proceeds as follows:

(1) All trees in a sample are decomposed into all their relevant partitions and the frequency of each partition is determined.

(2) A plot is made of equi-likelihood contours in the Q - S parameter space and the point of maximum likelihood is indicated by a cross.

(3) By a stepwise search accurate maximum likelihood values are determined for both the two-parameter Q - S space and for the one-parameter Q -space, at $S = 0$, and the one-parameter S -space, at $Q = 0$.

(4) The predictions made by means of the estimated parameter values are statistically tested, in all 3 cases, against the observed sample and the levels of significance obtained according to the procedure described above. The observed partition frequencies are also tested against the hypotheses of random terminal and segmental growth.

(5) A histogram plot is made of the ML partition probabilities for visual comparison with the observed partition frequencies.

(6) Each model (defined by Q and S) that predicts the sample in an acceptable way (level of significance greater than 0.05) is indicated by a dot in the parameter space.

Applications

Example 1

The data for this example come from the results of a study on pyramidal and multipolar non-pyramidal cell dendritic fields, kindly made available by McConnell

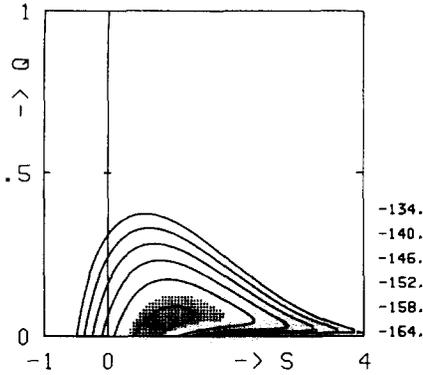


Fig. 1. Contours of equal likelihood for the sample of partitions obtained from basal dendrites of pyramidal cells in occipital cortex of 150-day-old control rats. The corresponding log-likelihood values are displayed right of the figure. The cross indicates the point of maximum likelihood $\hat{Q} = 0.028$ and $\hat{S} = 0.734$. The dotted area indicates all acceptable models (explain the partition frequencies in the sample of trees with a level of significance greater than 0.05). The maximum likelihood for the S -model is obtained for $\hat{S} = 0.598$ (not indicated in the figure).

and Uylings (in preparation). In this study the rats were of two age classes, and were maintained under several experimental nutritional and housing conditions. Twelve groups were distinguished. As an example the likelihood contours for the partition set derived from basal dendrites of pyramidal cells in occipital cortex of 150-day control rats are shown in Fig. 1. The maximum likelihood is obtained for $\hat{Q} = 0.028$ and $\hat{S} = 0.734$. The partition probabilities corresponding to these parameter values are displayed in Fig. 2, along with the observed frequencies, which are also compared with the predictions for the random segmental and terminal growth models. The discrepancy between these latter models and the observations is clearly shown. Both models differ significantly from the observations at a significance level < 0.001 . The two-parameter ML model, however, shows an agreement with the observations at a significance level of 0.765. Also, the one-parameter ML S -model ($\hat{S} = 0.598$) results in a good agreement at a significance level of 0.714. The dotted area, in Fig. 1, contains all acceptable growth modes. That means that all growth modes, outside the dotted area, are rejected by the goodness-of-fit test at a significance level of 0.05. This particular example shows that the growth of these branching patterns can be modelled by an order-dependent branching of predominantly terminal segments. Several conclusions can be drawn from the outcomes of all the groups.

(1) The a priori hypothesis of random terminal growth is rejected for 6 of the 12 groups of cells, while random segmental growth is rejected in all cases.

(2) The one-parameter Q -model is able to fit the data in an acceptable way (significance level > 0.05) for only 4 groups of cells.

(3) The one-parameter S -model is able to fit the data of all groups in an acceptable way.

(4) The two-parameter Q - S model is also able to fit the data of all groups in an acceptable way.

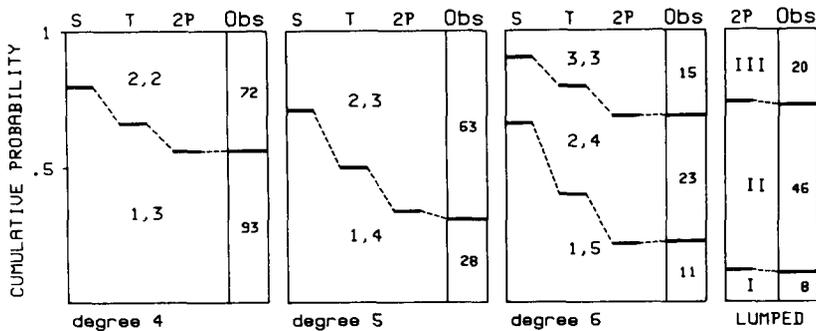


Fig. 2. Comparison of observed partition frequencies (Obs) from basal dendrites of pyramidal cells in occipital cortex of 150-day-old control rats and the partition probabilities predicted by the random segmental (S) and terminal (T) growth model and by the MLE values of the parameters for the two-parameter (2P) model. Both the random segmental and the terminal growth model predictions differ significantly from the observed data. The two-parameter ML model, however, corresponds very well to the observed data with a significance level of 0.765. Note that the sample also contains partitions of degree 7, 8, 9, 10, 11 and 13 but their numbers were too small for a χ^2 goodness-of-fit test. All these partitions are assigned to a composite distribution with class I containing all (1, $n-1$) partitions, class II all (2, $n-2$) partitions and class III the rest.

(5) The *S*-model describes a way of growth in which only terminal segments are allowed to branch while the branching probability of these segments is dependent on their order. Its MLEs are in all but two cases obtained for a positive value of the parameter *S*. This means that the terminal segments proximal to the soma have a higher probability to branch than the more distal ones. The two exceptions are cases where the *S* value, although slightly negative, does not differ significantly from random terminal growth ($S = 0$).

(6) The mean value for the *S*-model MLE is 0.65 ± 0.11 (S.E.M.) for all pyramidal cells and 0.24 ± 0.09 (S.E.M.) for all multipolar non-pyramidal cells. A *t*-test between both groups reveals that they are significantly different at a 0.02 significance level. These *S*-values appear to be correlated with the asymmetry of the partitions of the trees. Van Pelt and Verwer (1986) have studied the asymmetry properties of partitions and they presented in their paper contours of equal mean partition asymmetries in the *Q-S* parameter space. These figures show that contours for greater partition symmetry start at increasing *S*-values on the *S*-axis. The significantly higher *S*-value for the pyramidal cells may indicate that the partitions in these cells are significantly more symmetrical than those of multipolar non-pyramidal cells. Moreover, the positive *S*-values in all but two cases demonstrate that the partitions for all these groups are on the average more symmetrical than can be expected for trees grown by random terminal growth.

Example 2

The second example is an analysis of Purkinje cell dendritic trees taken from a study by McConnell and Berry (1978) on the effects of undernutrition on Purkinje cell dendritic growth in the rat. The authors have measured the dendritic networks

TABLE I

RESULTS OF THE ANALYSIS OF PURKINJE CELLS FROM 30-DAY-OLD RATS, REARED UNDER NORMAL AND DEPRIVED NUTRITIONAL SCHEMES (McCONNELL AND BERRY, 1978)

Rows a and b contain the results for the partition sets, derived from the actual subtrees of degree 4–6 from several Purkinje cells (Figs. 7 and 8 in McConnell and Berry, 1978). Rows c and d contain the results for single complete Purkinje cells (Fig. 4 in McConnell and Berry, 1978). The branching patterns are tested against the a priori hypothesis of random terminal growth and against the MLE results for the Q -model, S -model and Q - S model. The levels of significance are denoted by the letter P .

	Random terminal growth P	One-parameter models				Two-parameter model		
		Q -model ($S = 0$)		S -model ($Q = 0$)		Q - S model		
		\hat{Q}	P	\hat{S}	P	\hat{Q}	\hat{S}	P
Subtrees degree 4–6								
a, control	0.024	0.123	0.372	-0.186	0.369	0.267	0.625	0.216
b, underfed	0.000	0.289	0.647	-0.475	0.756	0.320	1.477	0.975
Complete tree								
c, control	0.243	0.102	0.373	-0.150	0.377	0.009	-0.125	0.264
d, underfed	0.694	0.000	0.485	-0.063	0.381	0.000	-0.063	0.164

in 30-day-old control and undernourished rats and have recorded the frequencies of peripheral subtrees of degree 4–6 from those networks. They have subsequently tested the subtree frequencies against the random terminal and segmental growth hypotheses by means of the χ^2 -test. Both control and undernourished groups appeared to disagree consistently with the segmental growth hypothesis. The random terminal growth hypothesis was also rejected for the undernourished groups and only accepted convincingly by the control group of degree 4. We have reanalyzed these data by means of the procedures described in this paper. The subtree frequencies recorded by McConnell and Berry (1978) are not independent from each other. For instance, recorded subtrees of degree 4 may very well originate from the recorded subtrees of degree 5 or 6. To avoid double counting we have only taken the first-order partitions of the recorded subtrees. The results for the random terminal growth hypothesis and the MLEs for the Q -model, the S -model and the Q - S model are presented in Table I (rows a, b). The levels of significance for the whole samples show that the random terminal growth hypothesis is rejected convincingly for the undernourished group and also rejected at a level of 0.024 for the control group. These findings are in agreement with the results of McConnell and Berry (1978) for the undernourished group. A comparison for the control group is unfortunately not possible because the 3 groups of degree, 4, 5 and 6 in the study of McConnell and Berry are not independent from each other and an overall result cannot be calculated. The outcomes for the ML models are in good agreement with the observed frequencies. For the control trees the 3 models differ little in their levels of significance. The likelihood landscape for the control group shows indeed a small gradient ridge which act as a centre of a band of acceptable growth models (level of significance greater than 0.05) (Fig. 3a). Note that although the ($Q = 0$,

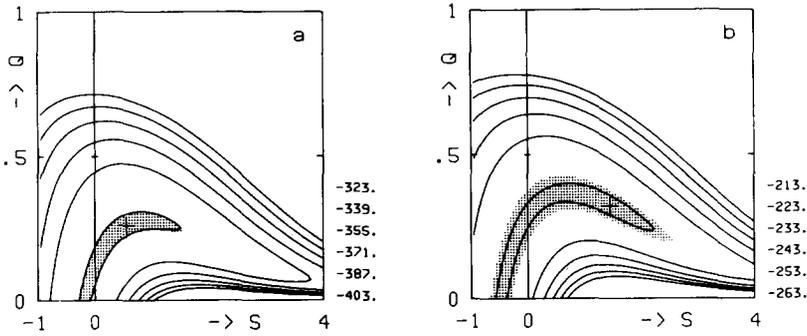


Fig. 3. Contours of equal likelihood for the set of partitions derived from peripheral subtrees of degree 4-6 in 30-day control (a) and undernourished (b) Purkinje cells (Figs. 7-8 in McConnell and Berry, 1978). The dotted area indicates all acceptable models (fitting the sample with a level of significance greater than 0.05). The likelihood landscape for the control rats is maximal for the S -domain at $\hat{S} = -0.186$, for the Q -domain at $\hat{Q} = 0.123$ and for the whole $Q-S$ domain at $\hat{Q} = 0.267$ and $\hat{S} = 0.625$ (cross). The corresponding values in the landscape for the undernourished cells are $\hat{S} = -0.475$, $\hat{Q} = 0.293$ and $\hat{Q} = 0.320$, $\hat{S} = 1.477$ (cross), respectively.

$S = 0$) point (random terminal growth) is situated just outside the ridge, it is already excluded by the band indicating a bad correspondence with the observations. The crossing of the band with the S - and Q -axis contains the one-parameter ML models. The likelihood landscape for the undernourished group is displayed in Fig. 3b. The agreement with the Q , S and $Q-S$ models is for this group also very good and illustrated further by the large band of acceptable models. Clearly both the random terminal and segmental growth modes are far outside the band and thus disagree strongly with the data. The perfect fit with the (Q, S) model is well demonstrated in Fig. 4 where the model outcomes are compared with the observed frequencies. A comparison of the results for the control and undernourished group reveals that the

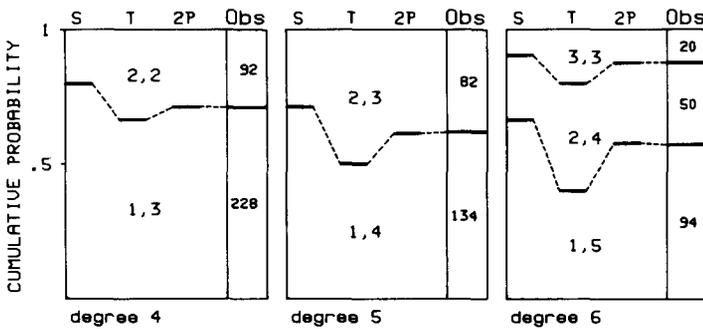


Fig. 4. Comparison of partition frequencies of Purkinje cell subtrees of degree 4-6 (Obs) from 30-day-old undernourished rats with partition probabilities predicted by the random segmental (S) and terminal (T) growth model and the two-parameter ML (2P) model. The predictions by both the random segmental and terminal growth model differ significantly from the observed data. The correspondence of the two-parameter ML model and the observed data is very good (level of significance is 0.975).

bands of acceptable growth modes for both groups do not overlap. This means that there is no growth mode which explains the partition frequencies of both groups satisfactorily and the control and undernourished samples must be regarded as significantly different. The same result was also obtained when the sets of partitions of both groups were tested directly (Verwer and Van Pelt, 1986). The band of acceptable models for the undernourished group is shifted into an upwards direction. Both ridges start at negative values on the S -axis, indicating that the mean partition asymmetries are greater than in the case of random terminal growth, while the partition asymmetry in undernourished dendrites is greater than in control dendrites. A direct comparison of the different growth modes in both groups is not possible because the formation of the trees is explainable by many growth modes (within the bands). Only if further assumptions are made can the comparison be more specific.

(1) If one assumes order independency in branching probabilities, then the MLE values $\hat{Q}(\text{control}) = 0.123$ and $\hat{Q}(\text{underfed}) = 0.289$ indicate that branching of intermediate segments occur more frequently in undernourished than in control cells.

(2) If, on the other hand, only branching of terminal segments is assumed, but allowance is made for order dependency, then the MLE values $\hat{S}(\text{control}) = -0.186$ and $\hat{S}(\text{underfed}) = -0.475$ indicate that: (a) in both groups the branching probability of terminal segments in the subtrees of degree 4–6 increases with their order; and (b) the order dependency is stronger in the undernourished cells.

Because our method is also applicable to single complete trees of any finite degree (greater than 3), we have analyzed the particular examples of complete Purkinje cell dendritic networks from 30-day control and undernourished rats given by McConnell and Berry (1978, their Fig. 4). From these beautiful camera lucida tracings we have counted all the partitions in both networks and the obtained partition frequencies were analyzed in the same way as described above. The results are presented in Table I (rows c, d). Both trees appear to correspond very well with the a priori random terminal growth hypothesis. The shape of the likelihood landscape for the control cell (Fig. 5a) appears to be considerably different from that of the peripheral-subtree partitions in Fig. 3a. The ridge-like feature is less clear and the area of acceptable models shows less extreme values for both parameters. The ML model for the complete tree is located precisely within the band for the peripheral subtrees. Moreover, both areas of acceptable models show considerable overlap which indicates that both the single complete Purkinje cell and the smaller subtrees from the periphery of many Purkinje cells can be described by the same growth modes. The likelihood landscape for the undernourished Purkinje cell (Fig. 5b) also shows a hill in a restricted area of the parameter domain with a small ridge-like feature on top of it. Also, the area of acceptable models is bounded by less extreme parameter values. A comparison with the corresponding area for the small subtrees reveals that there is no overlap at all, i.e. there is no model describing both the complete tree and the small subtrees in the underfed rats in an acceptable way.

The two-parameter ML model for the complete undernourished Purkinje cell ($\hat{Q} = 0$, $\hat{S} = -0.063$) indicates a growth mode close to random terminal growth. The

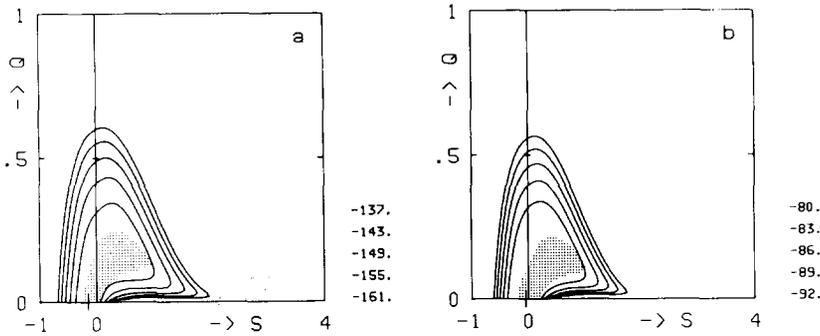


Fig. 5. Contours of equal likelihood for a complete control (a) and undernourished (b) Purkinje cell (McConnell and Berry, 1978, Fig. 4). The maximum likelihood for the control cell was found at $\hat{Q} = 0.009$, $\hat{S} = -0.125$ (cross) and for the undernourished cell at $\hat{Q} = 0$ and $\hat{S} = -0.063$ (cross). Note, that the crosses in the figures coincides with S -axes. The dotted area indicates all acceptable models (fitting the sample with a level of significance greater than 0.05).

small subtrees from the periphery of many of these Purkinje cells, however, are best described by substantial branching of intermediate segments and/or by order-dependent branching. Moreover, it is remarkable that the areas of acceptable models for both the control and undernourished complete cell show considerable overlap. If the analyzed Purkinje cells are representative for their groups, one can conclude from these findings that undernourishment manifests itself in different topological properties of only the subtrees at the periphery of the Purkinje cells which can be explained by significantly different growth modes.

Discussion

The peak in the likelihood landscape may indicate a growth mode which passes the goodness-of-fit test with the level of significance > 0.05 . Points in the vicinity of the peak, however, may also score better than 0.05 and must be regarded also as good model descriptions. The likelihood landscapes in Figs. 1 and 3a, b show that the peak is not isolated but is situated on a ridge along which the likelihood changes very slowly. The ridge starts at a certain point on the S -axis and bends in a positive S direction. The area of acceptable models also follows this ridge and is band-shaped. The crossing of the band over the S - and Q -axes indicates the models, which would have been obtained if the maximum likelihood search was done in only S - or Q -space. Generally, in modelling practice one tries to minimize the number of free parameters in order to optimize the information content of the model. In the case of a two-parameter model one may prefer a one-parameter approach on the basis of either additional assumptions or information and take the ML Q -model or the ML S -model. With respect to both one-parameter approaches, two situations may now occur.

(1) If the band starts at a positive S -value (Fig. 1), the ML S -model is then obtained for the MLE value of the parameter S . If the band and the Q -axis do not

have points in common, then the Q -model fails in producing an acceptable ML model.

(2) If the band starts at a negative S -value (Fig. 3a, b) and crosses the Q -axis, then both the Q -model and S -model produce MLEs for their parameters. Both approaches result in acceptable ML models and, without more information, one cannot make a choice between them. As a consequence, growth by branching of only terminal segments with increasing branching probabilities with centrifugal order ($Q = 0$, $S < 0$) can not be distinguished from growth by order-independent branching of both terminal and intermediate segments ($Q > 0$, $S = 0$).

The assumption of regularity at some level of description of the growth of branching patterns has been crucial in the mathematical modelling of growth and the development of statistical procedures. The observation that in all cases the model was able to predict the sample properties, at a high level of accuracy, must be regarded as strong support for this assumption and illustrates further that the model is well formulated. The incorporation of an order-dependency by the S -parameter has extended the range of predictable properties considerably. The application of the model to samples of dendritic trees has shown that in many cases a maximum likelihood estimate is only obtainable in S - or Q - S space. In view of these features, we can conclude that the model approach is a very fruitful one. The area of acceptable models characterizes a sample of trees and a comparison of areas for different samples of trees may reveal differences in their tree topologies. A comparison of samples of trees on the basis of either their partitions or other descriptive topological measures, however, may be much simpler and possibly preferable to detect differences, because it bypasses all the model calculations (Verwer and Van Pelt, 1986). Each topological measure will represent a part of the complete topological information contained in the ambilateral type frequency distribution. Methods for quantifying topology can thus only be compared with reference to their information content. Most (if not all) measures of topological properties appear to be dependent of the degree of the tree. This aspect may hinder an unambiguous interpretation of an averaged outcome for this measure of trees of different degrees. The growth model approach, on the contrary, has incorporated (formal) rules of development and as such its outcomes (maximum likelihood estimates of Q and S or areas of acceptable models) are independent of the degree distribution of the sample of trees.

A next question is how to interpret the results in more detailed biological terms. For this one has to note that the modelled regularity can be of global nature. For instance, tissue culture studies show that the outgrowth of dendrites is a very dynamic process and at a detailed time scale consists of extension and withdrawal of neurites and growth cones (Johnston and Wessells, 1980; Letourneau, 1982). Our model does not describe the growth at this timescale but may very well apply to a more global description in which only 'established' branches are taken into account. The occurrence of cut branches is another aspect not contained in the growth model. However, Van Pelt and Verwer (1984b) have shown that trees grown by random terminal or segmental branching, from which branches are removed at random, still agree in their topological properties with these models. If trees are

grown according to any other particular Q -model and branches are removed at random, then their topological properties appear to deviate from those of the uncut ones. These results (unpublished) show, however, that the difference between normal- and cut-tree partition distributions is very small and in practice negligible in comparison with the variation in observed sample frequency distributions.

Acknowledgements

We are grateful to A.J. Noest for his critical comments on the manuscript and G. van der Meulen for photography.

References

- Berry, M. and Flinn, R. (1984) Vertex analysis of Purkinje cell dendritic trees in the cerebellum of the rat, *Proc. R. Soc. London Ser. B.*, 221: 321–348.
- Cochran, W.G. (1954) Some methods for strengthening the common χ^2 tests, *Biometrics*, 10: 417–451.
- Johnston, R.N. and Wessells, N.K. (1980) Regulation of the elongating nerve fiber. In R.K. Hunt (Ed.), *Current Topics in Developmental Biology*, Vol 16, Academic Press, New York, pp. 165–205.
- Koziol, J.A. and Perlman, M.D. (1978) Combining independent chi-squared tests, *J. Am. Stat. Assoc.*, 73: 753–763.
- Letourneau, P.C. (1982) Nerve fiber growth and its regulation by extrinsic factors. In N.C. Spitzer (Ed.), *Neuronal Development*, Plenum Press, pp. 213–254.
- McConnell, P. and Berry, M. (1978) The effects of undernutrition on Purkinje cell dendritic growth in the rat, *J. Comp. Neurol.*, 177: 159–172.
- Van Pelt, J. and Verwer, R.W.H. (1983) The exact probabilities of branching patterns under terminal and segmental growth hypotheses, *Bull. Math. Biol.*, 45: 269–285.
- Van Pelt, J. and Verwer, R.W.H. (1984a) New classification methods of branching patterns, *J. Microsc.* (Oxford), 136: 23–34.
- Van Pelt, J. and Verwer, R.W.H. (1984b) Cut trees in the topological analysis of branching patterns, *Bull. Math. Biol.*, 46: 283–294.
- Van Pelt, J. and Verwer, R.W.H. (1985) Growth models (including terminal and segmental branching) for topological binary trees, *Bull. Math. Biol.*, 47: 323–336.
- Van Pelt, J. and Verwer, R.W.H. (1986) Topological properties of binary trees grown with order-dependent branching probabilities, *Bull. Math. Biol.*, 48: 197–211.
- Verwer, R.W.H. and Van Pelt, J. (1983) A new method for the topological analysis of neuronal tree structures, *J. Neurosc. Meth.*, 8: 335–351.
- Verwer, R.W.H. and Van Pelt, J. (1985) Topological analysis of binary tree structures when occasional multifurcations occur, *Bull. Math. Biol.*, 47: 305–316.
- Verwer, R.W.H. and Van Pelt, J. (1986) Descriptive and comparative analysis of geometrical properties of neuronal tree structures. In special issue 'Morphometry and Stereology in Neurosciences', *J. Neurosc. Meth.*, 18: 179–206.
- Verwer, R.W.H., Van Pelt, J. and Noest, A.J. (1986) Parameter estimation in the topological analysis of binary tree structures, *Bull. Math. Biol.*, submitted.
- Wonnacott, R.J. and Wonnacott, T.H. (1985) *Introductory Statistics*, Wiley, New York.