

## TERMINAL AND INTERMEDIATE SEGMENT LENGTHS IN NEURONAL TREES WITH FINITE LENGTH

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A basic but neglected property of neuronal trees is their finite length. This finite length restricts the length of a segment to a certain maximum. The implications of the finite length of the tree with respect to the segment length distributions of terminal and intermediate segments are shown by means of a stochastic model. In the model it is assumed that branching is governed by a Poisson process. The model shows that terminal segments are expected to be longer than intermediate segments. Terminal and intermediate segments are expected to decrease in length with increasing centrifugal order. The results are compared with data from *in vivo* pyramidal cells from rat brain and tissue cultured ganglion cells from chicken. A good agreement between data and model was found.

**1. Introduction.** The trees formed by dendrites of many neurons exhibit specific patterns of segment length distributions. One such feature is, with only a few exceptions (Uylings *et al.*, 1986), that on the average terminal segments are longer than intermediate segments of the same centrifugal order and that, even averaged over all orders, terminal segments are longer than intermediate segments (Ten Hoopen and Reuver, 1970; Uylings *et al.*, 1978; Connor *et al.*, 1982; Uylings *et al.*, 1986; Larkmann, 1991). The centrifugal order of a segment is equal to the number of branch points between the segment and the root of the tree and denotes the position of a segment in the tree. The difference in length between the two types of segments decreases with increasing order, mainly because terminal segment length decreases fast with increasing order, while intermediate segment length decreases slowly or remains constant. Longer terminal compared with intermediate segments are by no means a unique feature of neuronal trees, being also observed in the branching pattern of rivers (Smart, 1972; Smart and Werner, 1976).

When modelling segment lengths in structures like neurites, rivers, botanical trees and vascular systems two main approaches have been used. Scheidegger (1966), Ten Hoopen and Reuver (1971), Berry and Bradley (1976) and Buettner and Pittman (1991) use exponential distributions to describe the length

distributions of either intermediate segments alone or a combination of terminal and intermediate segments. Buettner and Pittman (1991) explain branching as a small but constant branch probability per unit length. The model of Nowakowski *et al.* (1990) uses the same approach, but in their case the branch probability is lowered for a while after branching has taken place.

As a second approach, Fisher and Honda (1979) and Crawford and Young (1990) explicitly formulate the relation between segment length and order in their models of outgrowth of botanical trees. The ratio between the length of the parent and the daughter branches is included as a parameter and is set to a certain value. In their model of Glasswort (*Salicornia* sp.) outgrowth, Ellison and Niklas (1988) use a similar approach. A length ratio to calculate the daughter segment length from the parent segment length is also used by Dawant *et al.* (1986) in their study of blood vascular systems.

Both approaches have their disadvantages when one wants to explain segment length distributions in neuronal trees. When a length ratio is used, the segment length distribution is part of the model and not a result. When exponential or exponential-like distributions are used, it is implied that with a low probability very long segments are able to occur. However, in reality the length of a segment is restricted to a maximum, because a tree has only a finite length.

The question addressed in this paper is how the finite length of the tree determines segment length distributions in neuronal trees. In the next section some basic segment length distribution properties will be stated in general terms. The case of outgrowth with a constant branch probability is further elaborated in section 3 and 4. The results of section 3 and 4 are compared with segment length distributions in neural dendrites from rat brain and tissue cultured neurites in section 8.

**2. General Considerations.** After a finite period of time a growing tree will have a finite length and terminal and intermediate segments can be distinguished. To analyse these trees for basic segment length patterns, we will confine ourselves to equal path lengths (that is the length of a path from the root to tip) and we will consider only the branch points along a single path from the root to a tip in a tree. The position of a segment on the path is given by the centrifugal order, in short "order", which is the number of branch points between the segment and the root of the path (Fig. 1).

Take the path length to be  $l_t$  at the time of observing and analysing the tree. If no branch points have occurred, the path contains only a terminal segment of order 0 and length  $l_t$ . If one branch event has occurred on the path, there will be an intermediate segment of order 0 and a terminal segment of order 1. The length from the root to the location of the first branch point is defined to be  $l_1$ , the length of the order 0 intermediate segment. If a path without a branch point

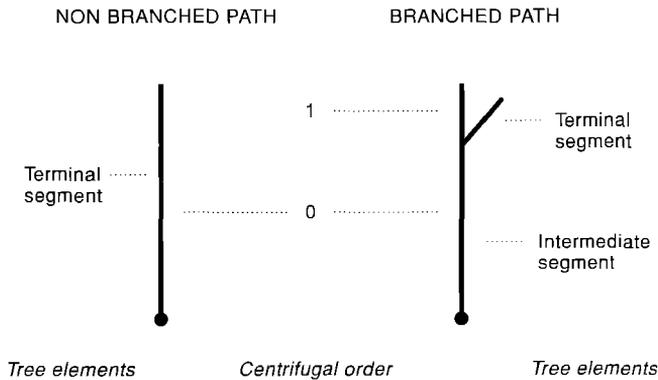


Figure 1. Elements of a nonbranched and a branched path of equal length.

is compared with any path containing one branch point, it will be clear that the terminal segment of order 0 is longer than or equal to the intermediate segment of order 0.

When  $n$  branch points occur on a path, the path contains  $n$  intermediate segments and one terminal segment. The length of the path from the root to the position of the  $i$ th branch point is defined to be  $l_i$ . The length of an intermediate segment of order  $i$  is then  $l_{i+1} - l_i$ , the length of the terminal segment, of order  $n$ , is  $l_t - l_n$ ,  $l_n$ , the length of the path from the root to the  $n$ th branch point. If, on a path containing  $n$  branch points, one additional branch event would occur, the path would contain an order  $n$  intermediate segment and an order  $n+1$  terminal segment. Because  $l_t \geq l_{n+1}$  and  $l_n$  is the same in both cases:  $(l_t - l_n) \geq (l_{n+1} - l_n)$ , the terminal segment of order  $n$  will be longer than (or at least as long as) the intermediate segment of that order.

The above must be interpreted in the sense that if an intermediate segment of order  $n$  occurs it will be shorter than or as long as the order  $n$  terminal segment would have been. Reconsidering the complete branched tree, this means that if, after a bifurcation, one daughter segment branches, while the other does not branch, the intermediate segment in the branched daughter is shorter than or equal to the terminal segment in the unbranched daughter.

These considerations also apply to terminal segment length. Take a path on which  $i$  branch events have occurred. The length of the terminal segment, of order  $i$ , is then  $l_t - l_i$ . If one additional branch event were to occur on the same path, then the length of the terminal segment, now of order  $i+1$ , would be  $l_t - l_{i+1}$ , because  $l_i \leq l_{i+1}$ ,  $l_t - l_i \geq l_t - l_{i+1}$ . This holds for every  $i$ . Again reconsidering a branched tree, it means that if one daughter branches at a bifurcation and the other remains unbranched, the terminal segment of the unbranched daughter is longer than or equal to the terminal segment of the branched daughter.

**3. Special Case: Constant Branch Probabilities per unit of Length.** In the foregoing general considerations the distribution and number of branch points was not specified. If the distribution can be specified, the expected lengths of intermediate and terminal segments can be calculated. In the present section the restriction to equal path lengths remains valid.

A simple stochastic process which describes the number and position of branch points on a path is a Poisson process. A Poisson process occurs when the probability that a branch event occurs within a small length interval is small, proportional to the length of the interval and constant for all, small, intervals with the same length (Cox and Lewis, 1966). When the branch events are given by a Poisson process and the total length of the path would be infinite, the length intervals between two subsequent branch events and the length intervals between a fixed point and the next branch event are exponentially distributed (Cox and Lewis, 1966; Stuart and Ord, 1987). For example, the length between root and first branch point is distributed as:

$$P(L_1 = l_1) = \beta e^{-\beta l_1} I_{(0, \infty)}(l_1) \quad (1)$$

where  $I_{(0, \infty)}(l_1)$  is the indicator function, which is 1 if  $l_1 \in [0, \infty)$  and 0 otherwise and  $\beta$  is a parameter, and  $E(L_1) = 1/\beta$ ,  $\text{var}(L_1) = 1/\beta^2$ . When a tree is observed at a certain moment, however, the total path length will necessarily be finite. Define this length to be  $l_t$ . The length intervals between the root and the first branch event are then distributed according to the conditional distribution  $P(L_1 = l_1 | l_1 \leq l_t)$ :

$$P(L_1 = l_1 | l_1 \leq l_t) = \frac{\beta e^{-\beta l_1}}{1 - e^{-\beta l_t}} I_{(0, l_t)}(l_1). \quad (2)$$

This is called the truncated exponential distribution. The length intervals are distributed according to a truncated distribution because the length interval between root and first branch point exists only if the first branch event has occurred. If the first branch event occurs, it has to occur at a length smaller than or equal to  $l_t$ , which explains the condition  $l_1 \leq l_t$ . The expected length between the root and the first branch event while the segment branches before or at  $l_1 = l_t$  is:

$$E(L_1 | l_1 \leq l_t) = \left[ \frac{1}{\beta} - \frac{l_t + 1/\beta}{e^{\beta l_t}} \right] \frac{1}{1 - e^{-\beta l_t}}. \quad (3)$$

The truncated exponential distribution gives the length distribution of the interval between the root and the first branch event, given that it occurs before or at  $l_t$ . The interval length between the root and the  $i$ th branch event, assuming that branch events are Poisson distributed along the path and that the path

length would be infinite, is distributed as a gamma distribution (Cox and Lewis, 1966; Mood *et al.*, 1974):

$$P(L_i=l_i) = \frac{\beta}{\Gamma(i)} (\beta l_i)^{i-1} e^{-\beta l_i} I_{(0,\infty)}(l_i) \tag{4}$$

with  $E(L_i)=i/\beta$  and  $\text{var}(L_i)=i/\beta^2$ . The truncated gamma distribution  $P(L_i=l_i|l_i \leq l_t)$ , correcting for a finite path length  $l_t$ , is given by:

$$P(L_i=l_i|l_i \leq l_t) = \frac{\beta}{\Gamma(i)} (\beta l_i)^{i-1} e^{-\beta l_i} \frac{I_{(0,l_t)}(l_i)}{1 - \sum_{k=0}^{i-1} \frac{e^{-\beta l_t} (\beta l_t)^k}{k!}} \tag{5}$$

For  $i=1$  the truncated gamma distribution simplifies to the truncated exponential distribution. The expected length from root to the  $i$ th branch event, while the  $i$ th branch event is located before or at  $l_i=l_t$ , is:

$$E(L_i|l_i \leq l_t) = \left[ \frac{i}{\beta} - \sum_{k=0}^i \left[ \beta^{k-1} l_t^k e^{-\beta l_t} \frac{1}{k!} \right] \right] \frac{1}{1 - \sum_{k=0}^{i-1} \frac{e^{-\beta l_t} (\beta l_t)^k}{k!}} \tag{6}$$

The variance of  $L_i|l_i \leq l_t$  can be calculated from:

$$\text{var}(L_i|l_i \leq l_t) = E(L_i^2|l_i \leq l_t) - (E(L_i|l_i \leq l_t))^2 \tag{7}$$

Its derivation is left to the reader. When the truncation length  $l_t$  is much larger than the expectation of  $L_i$ , the variance is approximated by  $i/\beta^2$ , the variance of the untruncated gamma distribution. This quantity increases with increasing branch event number  $i$ . However, when  $L_i$  becomes of the same magnitude as the truncation length, the truncation cuts with each increase in  $i$  a larger part of the tail at the right hand side of the untruncated gamma distribution, causing the variance to decrease.

**4. Segment Lengths.** The model as presented in the above is based on cumulative intermediate segment lengths. However, we are primarily interested in the individual intermediate and terminal segment lengths. These can be calculated from the model as follows:

*Intermediate segment length.* A segment is an intermediate segment when it ends in a branch point. Thus an intermediate segment of order  $i$  exists when an order  $i+1$  branch point is present. The expected length  $I_i$  of an intermediate segment of order  $i$  is:

$$I_i = E(L_{i+1}|l_{i+1} \leq l_t) - E(L_i|l_{i+1} \leq l_t) \tag{8}$$

where  $L_{i+1}|l_{i+1} \leq l_t$  is distributed as the truncated gamma distribution [equation (5)] and  $L_i|l_{i+1} \leq l_t$  is distributed as:

$$P(L_i = l_i | l_{i+1} \leq l_t) = \frac{P(L_i = l_i | l_i \leq l_t)P(l_{i+1} \leq l_t | l_i)}{\int_0^{l_t} P(L_i = l_i | l_i \leq l_t)P(l_{i+1} \leq l_t | l_i) dl_i} \tag{9}$$

where

$$P(l_{i+1} \leq l_t | l_i) = \int_0^{l_t - l_i} \beta e^{-\beta L} dL = 1 - e^{-\beta(l_t - l_i)}$$

and  $L$  an integration variable.

*Terminal segment length.* A terminal segment of order  $i$  is present when the  $i$ th branch point does exist, but the next one does not. For terminal segments,  $l_i$  will be distributed as:

$$\begin{aligned} P(L_i = l_i | l_i \leq l_t, l_{i+1} > l_t) &= \frac{P(L_i = l_i | l_i \leq l_t)P(l_{i+1} > l_t | l_i)}{\int_0^{l_t} P(L_i = l_i | l_i \leq l_t)P(l_{i+1} > l_t | l_i) dl_i} \\ &= \frac{l_i^{i-1}}{l_t^i} \end{aligned} \tag{10}$$

where  $P(L_i = l_i | l_i \leq l_t)$  is given by equation (5) and  $P(l_{i+1} > l_t | l_i) = 1 - P(l_{i+1} \leq l_t | l_i)$ . The expected length  $T_i$  of a terminal segment of order  $i$  becomes:

$$\begin{aligned} T_i &= l_t - E(L_i | l_i \leq l_t, l_{i+1} > l_t) \\ &= \frac{1}{i+1} l_t. \end{aligned} \tag{11}$$

**5. Segment Length Properties.** With the aid of the formulated probability density functions and the expectations, the general considerations can be extended in the following way:

- (1) Terminal segments decrease in length with increasing order. The expected terminal segment length  $T_i$  is a monotonic decreasing function of order  $i$  [equation (11)].
- (2) The expected length of intermediate segments decreases with increasing order. It can be proven that (Appendix):

$$E(L_1 | L_1 \leq b) > E(L_1 | L_1 \leq c) \quad \text{if } b > c. \tag{12}$$

Consider an intermediate segment of order  $i$ . The expected length of the interval between branch event  $i$  and  $i+1$ , the expected length of the intermediate segment, is given by  $E(L_1 | L_1 \leq l_t - l_i)$ . The expected length

of the next intermediate segment, in between branch events  $i + 1$  and  $i + 2$ , is given by  $E(L_1 | L_1 \leq l_t - l_{i+1})$ . Because  $l_t - l_i > l_t - l_{i+1}$  within every path, regardless of the exact positions, we can use inequality (12), and inequality  $E(L_1 | L_1 \leq l_t - l_i) > E(L_1 | L_1 \leq l_t - l_{i+1})$  holds. Because this does not depend on the actual value of  $i$ , it holds for every pair of consecutive intermediate segments. The expected lengths of intermediate segments will therefore decrease with increasing order.

- (3) Due to the complicated form of  $E(L_i | l_{i+1} \leq l_t)$ , it is difficult to compare lengths of intermediate and terminal segments of the same order in an analytic way. When the parameters  $\beta$  and  $l_t$  are estimated from real data (see section 6 for details), intermediate segments are smaller than terminal segments (Fig. 2).
- (4) Averaged over all orders, the length of terminal segments is, at least at low branch probabilities, longer than the length of intermediate segments. The expected length of segments averaged over all orders depends on the expected segment length per order and the frequency distribution of the number of segments per order. For terminal segments this expected length is given by:

$$E(l_t - L) = \sum_{j=0}^{\infty} T_j P(J=j) \tag{13}$$

where

$$P(J=j) = \frac{e^{-\lambda} \lambda^j}{j!}$$

and  $L$  denotes the path length to the last branch point,  $E(l_t - L)$  the expected length of a terminal segment averaged over all orders,  $T_j$  the expected length of a terminal segment of order  $j$ , and  $P(J=j)$  the probability that the path contains only  $j$  branch points, with  $\lambda$  the mean number of branch points per path.  $P(J=j)$  has the same distribution as the distribution of the number of branch events within the interval  $l_t$ . This distribution is a Poisson distribution under the assumptions made here. The expected length of an intermediate segment averaged over all orders is given by:

$$E(D) = \sum_{k=0}^{\infty} I_k P_K(K=k) \tag{14}$$

where  $E(D)$  the expected distance between two consecutive branch points, that is the length of an intermediate segment,  $I_k$  the expected length of an intermediate segment of order  $k$  and  $P_K(K=k)$  the probability of occurrence of an intermediate segment of order  $k$ . This

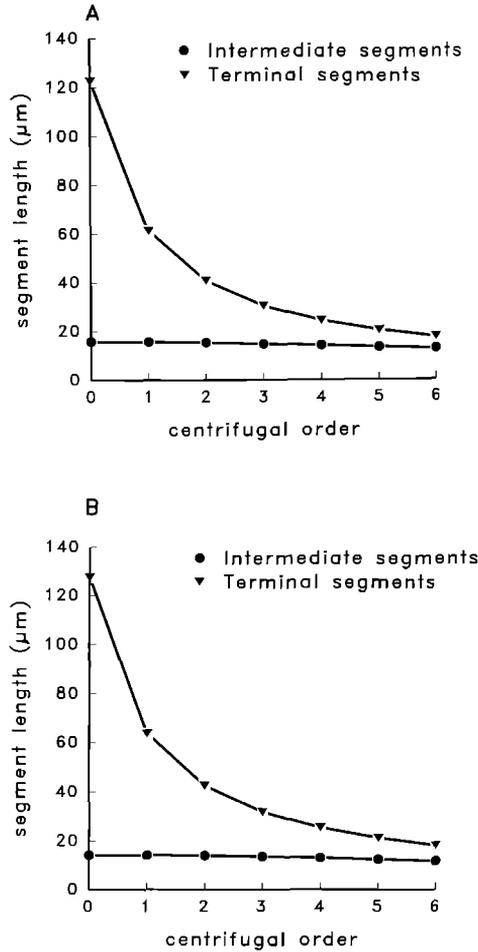


Figure 2. Segment length–centrifugal order relation as predicted from the model with parameters estimated from the data of McConnell and Uylings, length class 120–125 μm (A) and length class 125–130 μm (B).

distribution is more complicated than a Poisson distribution, because after the occurrence of  $j$  branch events on a single path,  $j - 1$  intermediate segments are present, which are all taken into account. The probability of occurrence of an order  $k$  intermediate segment is thus given by:

$$P_K(K=k) = \sum_{j=k+1}^{\infty} P(J=j) \tag{15}$$

where  $P(J=j)$ , the probability that exact  $j$  branch points occur on the path, is given by the same Poisson distribution as used with terminal segments. Equations (12) and (13) could not be solved in analytic form,

but can be calculated numerically when all parameters are known. When this is done using the parameters  $\beta$  and  $l_t$  estimated from real trees (see next section for details) and taking the parameter  $\lambda$  in the Poisson distribution equal to the mean number of branch events per path, it appears that the expected terminal segment length is about 3.5 times as long as the expected intermediate segment length (Table 1).

Table 1. Mean number of branch events, expected lengths of terminal and intermediate segment lengths, irrespective of order, based on parameters estimated from the data of McConnell and Uylings (see Table 2, without branchless period)

Dataset	Mean number of events	Expected terminal segment length	Expected intermediate segment length
120–125 $\mu\text{m}$	2.16	50.21 $\mu\text{m}$	14.09 $\mu\text{m}$
125–130 $\mu\text{m}$	2.25	50.69 $\mu\text{m}$	15.57 $\mu\text{m}$

This result can be verified with the results of item 3 of this section. There it appeared that with the parameters estimated from real trees, terminal segments are longer than intermediate segments at least up to an order of 6 (Fig. 2). These are the orders with the dominant frequency of occurrence. Because up to an order 6 *all* expected terminal segment lengths are longer than the expected intermediate segments lengths, it is not striking that also on average terminal segments are longer than intermediate segments.

**6. Segment Length Frequency Distributions.** The truncated gamma distribution implies a monotone decreasing truncated exponential distribution of the length intervals between two consecutive branch events. The observed distributions, however, are not monotone decreasing, but unimodal and highly skewed to the right (Berry and Bradley, 1976; Uylings *et al.*, 1978; Nowakowski *et al.*, 1990). Avoiding complicated functions (Nowakowski *et al.*, 1990), this segment length distribution can be approximated by adding, after each branch event, an interval in which no branch events occur. The length of this branchless interval is denoted by  $\tau$  and accounts for a minimal length for each segment. The path length in which branch events are able to be located is then given by:

$$l^* = l_t - i\tau \quad (16)$$

where  $l_t$  is the path length,  $\tau$  the length of the branchless interval and  $i$  the number of branch events which are located within  $l_t$ . The probability density

function of a truncated distribution with branchless period is the same as given by equation (3) and equation (5), but with the random variable  $L_i$ , the length until branching for the  $i$ th time transformed to  $L_i^* = L_i - i\tau$ , the realized value  $l_i$  of  $L_i$  transformed to  $l_i^* = l_i - i\tau$  and the path length transformed to  $l_i^* = l_i - i\tau$ . By putting  $i$  instead of  $i + 1$  in the transformation, it is implied that no branchless interval is present after the root.

**7. Unequal Path Lengths.** The restriction to equal path lengths can be relaxed to the more general situation of unequal path lengths. This is the situation actually encountered in neuronal trees, where the path lengths will differ for the paths within a tree and between trees. The model as presented so far predicts the intermediate and terminal length distributions given a certain path length:  $P(l_i|l_i)$ ,  $l_i$  referring to lengths of either intermediate or terminal segment of order  $i$  [equations (9) or (11)]. To incorporate unequal path lengths, the joint probability density of segment lengths and path lengths is calculated:

$$P(l, l_i) = P(l|l_i)P(l_i)$$

where  $P(l_i)$  denotes the distribution of path lengths. Integration from  $l_i = 0$  to  $\infty$  results in the unconditional length distribution  $P(l)$ . In this approach it is assumed that the distribution of the path lengths  $P(l_i)$  is known. If it is not known, the observed path length distribution is taken and:

$$P(l) \approx \sum_{j=1}^n P(l|l_{t,j})F(l_{t,j})$$

where  $l_{t,j}$  the  $j$ th path length bin,  $j = 1 \dots n$ ,  $n$  the total number of bins and  $F(l_{t,j})$  the fraction of path lengths in the  $j$ th path length bin.

The extension of the model to unequal path length implies that the variance of the segment length distribution at a particular order increases, as can be seen from the multiplication with the path length distribution in equation (17). The source of the additional variance introduced by analysing unequal path lengths can be illustrated with Fig. 2 as well. In Fig. 2 the expected segment lengths versus order for two path lengths are shown. These expected lengths do not overlap, the segment lengths belonging to the longer path length being somewhat longer than those belonging to the shorter path length (see for instance the length of zero order terminal segments). Due to this difference, variance is added to the mean value if the segments lengths from the two path lengths are averaged.

**8. Comparison with Neuronal Trees.** To test whether patterns described by the truncated gamma distribution apply to real dendrites, the segment lengths of tissue cultured neurites (a general term for both axons and dendrites)

presented by Bray (1973) and unpublished basal dendrites of pyramidal cells from the cortex of rat (measured by P. McConnell and kindly made available by H. Uylings) were analysed. These neuronal trees pose one problem not mentioned yet. The paths within a single tree share at least the root segment with each other. This implies that the segment lengths per order are not independent for the paths within a tree. For instance, the root segment is shared by all paths and has the same length in all paths. To avoid this dependence, only the segment lengths along one path per dendrite from the root to a randomly chosen terminal tip were measured for each neurite.

To avoid the extra variance introduced by unequal path lengths, we confined ourself to paths belonging to a small path length class. This way the implications of the finite path length for the intermediate and terminal segment lengths can be shown more clearly. Therefore, in the data of McConnell and Uylings only those paths were used that fall in the length classes 120–125  $\mu\text{m}$  and 125–130  $\mu\text{m}$ . Both classes are analysed separately. In case of the data of Bray the path lengths used in the analysis ranged from 145–225  $\mu\text{m}$  with a mean of 169.5  $\mu\text{m}$ . Because of the small number of neurites, no further division in length classes was made.

The parameter  $\beta$  from the truncated gamma distribution and the length of the branchless period  $\tau$  were estimated by maximum likelihood from the data of the intermediate segment lengths. In case of the data from Bray (1973)  $l_t$  was estimated from the mean length of two unbranched neurites. In case of the data of McConnell and Uylings, the path length  $l_t$  was estimated per length class by the mean path length.

The data of Bray (1973) are presented in Fig. 3 and the results for the best fitting model are shown in Fig. 4. The data of McConnell and Uylings are given in Fig. 5, the results for the best fitting model are shown in Figs 2 and 6. The estimates of  $\beta$ ,  $\tau$  and  $l_t$  are given in Table 2. In both datasets it is clear that terminal segments are longer than intermediate segments of the same order. Terminal segments decrease in length with increasing order. The intermediate segments tend to have a constant length over all orders.

The models with and without branchless period both give a good fit to the data, Figs 4 and 6. All datasets show large variances, but this variance is of the same magnitude as the expected variance. The variance predicted from the model with branchless period is slightly smaller than the variance predicted from the model without branchless period. The predicted mean values of both models are quite the same. From Fig. 3 it appears that the intermediate and terminal segment lengths as a function of order in the data of Bray (1973) match the model well. Terminal segment length is a steeply decreasing function of order, as predicted by the model. In the data of McConnell and Uylings (Fig. 5) the terminal segment length is not as steeply decreasing in the model.

The mean intermediate and terminal segment lengths averaged over all

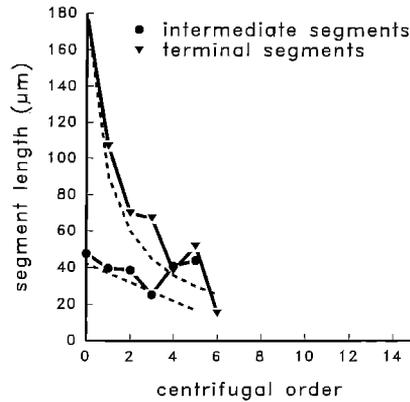


Figure 3. Segment length-centrifugal order relation of the neurites depicted by Bray (1973). The dotted line denotes the predicted values.

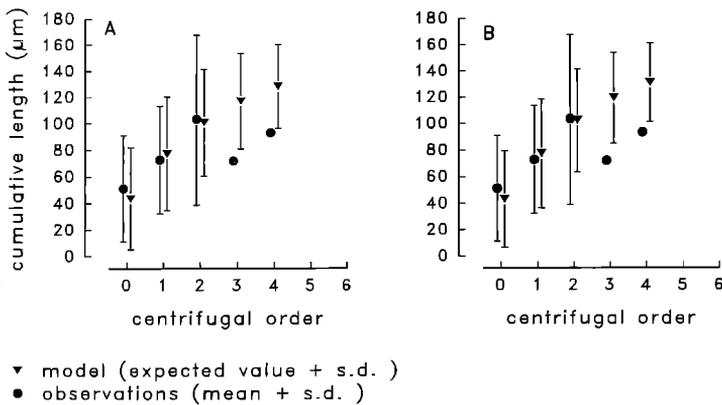


Figure 4. Cumulative intermediate segment length and its standard deviation as a function of order in the neurites of Bray (1973) and the same from the model fitted to the observations. (A) Model without branchless interval, (B) model with branchless interval.

orders from the data of McConnell and Uylings are given in Table 3. As predicted from the model with the parameters  $\beta$ ,  $l_t$  and the mean number of branch points  $\lambda$  estimated from the data (Table 1), terminal segments are much longer than intermediate segments.

These results show that the segment length versus order relations as expected from the truncated gamma distribution correspond to distributions found in neuronal dendritic trees. The addition of a branchless period does not improve the fit of the model.

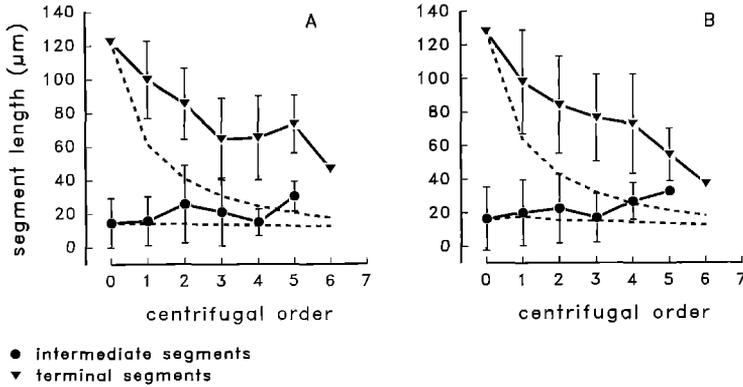


Figure 5. Segment length–centrifugal order relation in the neurites of McConnell and Uylings. (A) length class 120–125  $\mu\text{m}$ ; (B) length class 125–130  $\mu\text{m}$ . The dotted line denotes the predicted values.

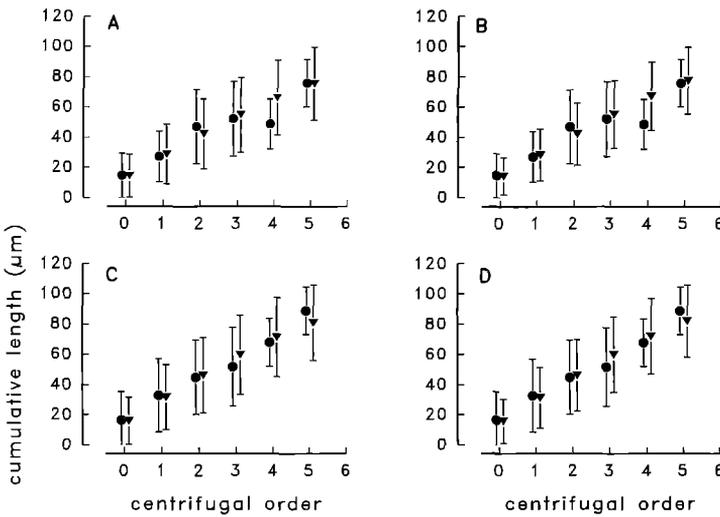


Figure 6. Cumulative intermediate segment length and its standard deviation as a function of order in the neurites of McConnell and Uylings and the same data from the model fitted to the observations. (A) Model without branchless interval, path length 120–125  $\mu\text{m}$ ; (B) model with branchless interval, path length 120–125  $\mu\text{m}$ ; (C) model without branchless interval, path length 125–130  $\mu\text{m}$ ; (D) model with branchless interval, path length 125–130  $\mu\text{m}$ . ( $\blacktriangledown$ ) expected value  $\pm$  standard deviation, ( $\bullet$ ) observed mean value  $\pm$  standard deviation.

**9. Discussion.** Although the finite length of a neuritic tree seems a trivial observation, it could be shown that it is a primary determinant of intermediate and terminal segment length distributions. In fact the whole notion of “terminal segment” is only valid in trees of finite length. From the general

Table 2. Estimated values of  $\beta$ ,  $\tau$  and  $l_t$  in models with and without branchless interval

Data	Model without branchless interval		Model with branchless interval		
	$\beta$	$l_t$	$\beta$	$\tau$	$l_t$
Bray (1973)	0.021	174.0	0.023	2.4	174.0
McConnell and Uylings					
120–125 $\mu\text{m}$	0.070	122.6	0.081	1.8	122.6
125–130 $\mu\text{m}$	0.063	127.5	0.068	1.0	127.5

Table 3. Observed lengths of terminal and intermediate segments in the data of McConnell and Uylings

Dataset	Observed terminal segment length	Observed intermediate segment length
120–125 $\mu\text{m}$	84.64 $\mu\text{m}$	17.60 $\mu\text{m}$
125–130 $\mu\text{m}$	85.69 $\mu\text{m}$	18.86 $\mu\text{m}$

considerations two properties could be deduced which always occur in branched trees with identical path lengths within the tree. The first is that, if at a bifurcation one daughter branches and the other does not branch, the terminal segment of the unbranched daughter is longer than or equal to the intermediate segment of the branched daughter. Secondly, the terminal segment of the unbranched daughter is also longer than or as long as the terminal segment of the branched daughter.

Assuming a Poisson process to determine number and position of branch events, it could be shown that terminal segments are on the average longer than intermediate segments of the same order. With parameters derived from *in vivo* and *in vitro* grown dendrites it could even be shown that, averaged over all orders, terminal segment length is longer than intermediate segment length. The inclusion of a branchless interval after each branch event did not change these patterns. For the description and interpretation of morphological data the foregoing implies that: (1) models describing segment lengths should take the finite length of a tree into account and (2) no complicated processes, i.e. changes in branch probability, have to be assumed to generate these patterns.

The model generates segment length versus order patterns based on the simple assumption of a Poisson process, in which each piece of the path has the same branch probability. The segment length patterns generated by the model therefore represent the basic patterns expected from such a Poisson branch

process. These patterns can be used for comparison with observed patterns from *in vivo* or *in vitro* dendrites and axons. If these dendrites and axons exhibit branching not based on a Poisson process, that is the branch probability is not constant along the path, their segment length patterns should deviate from the ones generated by the model. In this way, the model can be used to distinguish between the simple patterns generated by a Poisson process and other patterns, which may be formed by a more complicated branching process.

A biological situation in which a Poisson process could occur is the case of a constant growth rate and terminal growth and branching of the outgrowing neurite. If the branch probability per unit of time is constant, the branch process can be interpreted as outgrowth with a constant branch probability per unit of length at the tips of the outgrowing neurite. During each fixed time interval the path is extended by a fixed length and within that period there is a small probability that a branch event occurs. The neurites described by Bray (1973) exhibit a constant growth rate and terminal growth and branching. Their similarity with the model predictions suggests that their branch probability per time is indeed constant.

The segment length structure as modelled here will be most prominent when the total number of branch events is small, or when there is interest in the top of a growing tree. If, in contrast, the number of branch events is large, the part that separates the truncated distribution [equation (4)] from the untruncated one [equation (5)] is close to unity, and truncation does not have much effect. In that case all intermediate segments have the same expected lengths. Only for segments with a high order, at the very top of the tree, the truncated distribution will differ from the untruncated one. The situation of a limited number of branch events certainly holds for the neural trees used in the examples. Another situation in which a limited number of branch events is present are certain river networks. Howard (1971) and Smart (1972) show that, as predicted from the present model, terminal segments are longer than intermediate segments, irrespective of centrifugal order.

In contrast to rivers and the neural trees described here, botanical trees and blood circuitry vascular systems have many more branch events occurring during outgrowth. Except for the top of the structure, the truncation effect will not contribute much to the segment length structure of these trees. In the case of botanical trees the assumption of a Poisson process for describing number and position of branch events on a path in the tree is probably violated too. For example the first branch event of *Tabebuia rosea*-trees seems to be coupled to the length of the trailing segment (Borchert and Tomlinson, 1984). In the later stages of outgrowth arrested buds are reactivated. At this location multifurcations can be formed, which are not taken into account by the model presented here. Another example is Glasswort (*Salicornia europea*), which is only able to branch in between the basic elements of the stem, called internodia (Ellison and

Niklas, 1988). Two subsequent branch events are thus always separated by a minimal one internodal length.

Although the model is able to explain much of the segment length distributions of neuronal trees as a result of the finite path length, two aspects of the observed distributions are not covered by the model predictions. Firstly, the model predicts terminal segment lengths which are too small compared to the data of McConnell and Uylings. In contrast, the terminal segments lengths in the data of Bray are predicted nicely by the model. The dendrites measured by McConnell and Uylings stem from (nearly) mature cells. The neurites from Bray are all very young, in their starting phase of outgrowth. The neurites measured are thus from different developmental stages. It might be that during development the growth process changes. The data of McConnell and Uylings are consistent with the hypothesis that, at some point in outgrowth, the branch probability of terminal segments decreases, resulting in terminal segments which are too long compared to the predictions of the Poisson process. In both the very young neurites and the mature dendrites, the model predicts that the average terminal segment length is longer than the average intermediate segment length, as actually observed in the data.

A second deviation from the implications of the model is the length frequency distribution of segments from a single order. The present model implies a monotonic decreasing distribution or a distribution with a fixed minimal length if a branchless period after branching is assumed. Experimental data show an unimodal distribution, with the mode close to zero and a long tail to the right. More complicated functions of the branch probability after branching (as used by Nowakowski *et al.*, 1990) render segment length distributions much like the ones found in real neural trees.

In conclusion, by only assuming a Poisson process for describing the occurrence of branch events and terminal growth and branching, it could be shown that much of the segment length structure as observed in neuronal trees emerges from the model. No additional parameters, such as parent–daughter length ratios or a nonconstant branch probability, were needed to reach these results. Much of the structure is caused primarily by the finite path length of trees at the time of observation. This includes the large variances of the intermediate segment lengths, as observed in neural trees.

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## APPENDIX

Prove that  $E(L_1/L_1 \leq b) > E(L_1/L_1 \leq c)$  if  $b > c$ . Writing out the expectations according to equation (3):

$$\left[ \frac{1}{\beta} - \frac{b+1/\beta}{e^{\beta b}} \right] \frac{1}{1-e^{-\beta b}} > \left[ \frac{1}{\beta} - \frac{c+1/\beta}{e^{\beta c}} \right] \frac{1}{1-e^{-\beta c}} \quad (\text{A1})$$

this can be rewritten as:

$$\frac{c e^{\beta b - 1}}{b e^{\beta c - 1}} > 1. \quad (\text{A2})$$

If  $c = b$  then the left hand side of (A2) is equal to 1. If we define  $b = c + \delta$ , where  $c$  has a constant value and  $\delta$  is the variable, and if we define the left hand side of (A2) as  $f(\delta)$ , it appears that:

$$\frac{d}{d\delta} f(\delta) > 0. \quad (\text{A3})$$

Because  $f(\delta=0) = 1$  and the value of  $f(\delta)$  increases with increasing  $\delta$ , (A2) is true and (A1) holds.

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