A Model for Outgrowth of Branching Neurites

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The outgrowth of branching neurites is studied by modeling the dynamic behavior of the growth cones, which are the active structures at the neuritic tips. The basic actions of the growth cones comprise elongation, branching and guidance as a result of interaction with the local environment. The neurite itself is modeled by an elastic structure. The model is able to reproduce metrical and topological characteristics of tissue-cultured neurites (described by Bray, 1973, J. Cell Biol. 56, 702-712). The model shows that morphological characteristics of neurites can emerge from mechanisms acting purely at the level of the growth cone.

1. Introduction

During their outgrowth neuronal dendrites and axons, in general called neurites, attain a morphology typical for the type of neuron from which they stem. The quantification of the morphological characteristics of a dendrite or axon is one of the major objectives in the field of quantitative neuroanatomy. Morphological characteristics include the lengths of segments, the angles between the segments at a bifurcation, and the space filling of the dendrite. Because of the complex three-dimensional structure, many measures and procedures have been proposed for quantification (e.g. Uylings et al., 1989a,b). Neuritic morphology itself is the result of a developmental process, going on during the whole life span of an animal.

A basic question is which factors play a key role in the generation of the morphological characteristics. Tissue culture experiments have established the crucial role of the flattened tips of the neurites, the growth cones, in the outgrowth (Wessells & Nuttall, 1978; Lockerbie, 1987). These growth cones appear to be dynamic structures which, in interaction with their local environment, propagate and branch. It is therefore assumed that the dynamic action of growth cones is the primary determinant of the eventual morphology of dendrites and axons. However, the translation of detailed growth cone dynamics to dendritic and axonic morphology is not a trivial task. A modeling approach for studying the relation between the “microscopic dynamics” of the growth cones and the “macroscopic morphology” of the neurite can be fruitful. It allows for a systematic study of this complex system.

To study non-branching axonal guidance Gierer (1981), Bonhoeffer & Gierer (1984), Fraser (1980) and Katz & Lasek (1985) have presented models based on environmental heterogeneity. Heterogeneity is introduced by patterns in the substrate.
on which the axons grow. Modeled growth cones determine the direction of propagation by sampling environmental cues. These cues guide the growth cones of the axons along stereotype pathways.

A particular class of models describing neuronal outgrowth consider only topological aspects, based on the connection patterns of segments and bifurcation points (e.g. Van Pelt & Verwer, 1985; Horsfield et al., 1987). Metrical aspects such as lengths and angles are ignored. These models are able to explain the observed topological variance in dendritic trees (Van Pelt et al., 1989a).

Both axonal guidance models and topological models consider only a part of neuronal geometry, the former discards branching, while the latter discards the metrical aspects of a tree. To incorporate all geometrical aspects into the process of outgrowth of a single neurite a new metrical model has been developed. It describes outgrowth by modeling the actions of the individual growth cones in the neuritic tree. We will confine ourself to a single neurite and growth on a two-dimensional environment and study in a general way how growth cone behavior and neuritic morphology are causally related.

In section 2 current ideas and experiments on growth cone behavior are described. In section 3 the building blocks of the model are presented, based on the biological concepts given in section 2. The general properties of the model are described in sections 4 and 5. In section 6 the outcomes are compared with tissue-cultured neurites.

2. Neuritic Outgrowth

Growth cones are visible on most tips of growing dendrites and axons and consist of a central part from which filopodia and lamellipodia emerge. At the growth cone the insertion of material into the neurite occurs (Goldberg & Burmeister, 1989; Gordon-Weeks, 1989). Tissue culture experiments with growth cones disconnected from the cell body showed that they are able for some time to elongate the piece of neurite behind them (Shaw & Bray, 1977).

The filopodia and lamellipodia adhere to the surface on which they are growing (Letourneau, 1975, 1979). This active attachment depends on ligand–receptor binding and is thought to have a dominant influence in guiding the growth cone (Dodd & Jessell, 1988; Turner & Flier, 1989). Guidance of the growth cone would occur when the ligands for which the growth cone has receptors are dispersed in a gradient and the growth cone detects different densities. Besides adhesion a number of other influences on the direction of growth have been described. Electric fields and gradients in chemical compounds like neural growth factor change the direction of propagation of a growth cone (Purves & Lichtman, 1985; McCaig, 1990). During growth, the growth cone stays in line with the trailing segment. Shifting the segment will change the direction of propagation. Experiments by Bray (1979) clearly show this kind of behavior.

Filopodia are thought to sense these guiding cues (O'Connor et al., 1990). It is hypothesized by several authors that the filopodia exert mechanical force to the growth cone (Bray, 1979, 1987; Letourneau, 1982; Turner & Flier, 1989). The tension
in the segment behind the growth cone is, at least in part, attributed to growth cone movement (Lamoureux et al., 1989).

Vaughn et al. (1974) hypothesized that branching of the growth cone is also mediated by the filopodia. It would occur when two filopodia of the same growth cone simultaneously detect axon endings. This hypothesis was later extended to include more general adhesion (Berry & Bradley, 1976). The general idea behind these thoughts was confirmed by Wessells & Nuttall (1978) but they showed that not merely the adhesion of the filopodia, but the distribution of the adhered filopodia determined branching. This is consistent with the hypothesis of Bray (1979).

The neurite itself is observed to be an elastic strut (Dennerl et al., 1988; Lamoureux et al., 1989). This structure will be adhered to the substrate at the tips where the growth cones are present. In contrast to the adherence of the growth cones, the amount of adherence of the cell body, at the root of the neurite, depends on the kind of substrate used. In the case of uncoated glass, Bray (1970, 1973) describes that the cell body is loose from the substrate. In the case of laminin coating, the experiments of Zheng et al. (1991) show that the cell body is adhered to the substrate. The data of Bray (1973; figures 6–9) show that, when two neurites extend from the same loose cell body, the movement of the cell body is small compared to the elongation rate of the neurites.

3. Building Blocks of the Model

The experiments mentioned in the former section suggest a central role for the growth cones in three kinds of actions: (1) propagation of the growth cone and elongation of the trailing segment; (2) changing the direction of propagation; and (3) branching into daughter branches each with its own growth cone. The last two actions depend on the state of the local environment as detected by the growth cone.

The aim of the present modeling study is to formulate these concepts into a mathematical model which can be used to study how growth cone behavior and neuritic morphology are related. In the present section the building blocks of the model will be given. In section 4 properties which can be derived from the model structure within a single building block are given. Section 5 combines all building blocks into one simulation model and properties of the modeled neurites are given.

3.1. THE GROWTH CONE

Real growth cones consist of a central area from which filopodia emerge. The filopodia are able to reach a limited area near the growth cone. In the model the growth cone is defined by a detection area and filopodial vectors (Fig. 1). The radius of the modeled growth cone, that is the radius of the detection area, includes the central area and the filopodia of the real growth cone. Within the detection area of the modeled growth cone, all adhesion sites on the substrate will support a filopodial vector. Although under experimental circumstances many potential adhesion sites will be present, in the model only those adhesion sites which will support a filopodial vector are considered.
The detection area is defined as a circle with a cut part, where the trailing segment is attached to the growth cone. In this part no adhesion sites can be detected. The opening angle $\phi_n$ is the total angular width of the detection area.

When the adhesion sites in the detection area have been found, filopodial vectors are drawn from the center of the circle in the direction of the adhesion sites. The subsequent direction of propagation of the growth cone is given by the vectorial sum of the equally weighted filopodial vectors.

3.2. THE NEURITIC TREE

All segments of the neurite are subjected to elastic deformation, as shown by experimental data. Because the neurite is in rest when no growth cones actually grow, a force equilibrium must be present. Dennerl et al. (1988) have shown that unbranched neurites behave like simple elastic strings with an elastic tension proportional to the relative stretching. In this case the elastic tension $f_j$ in segments $j$ ($j = 1, \ldots, m$, $m =$ number of segments) is given by:

$$f_j = \frac{c \cdot A_j}{l_{ij}} (l_j - l_{ij}) = c \cdot A_j \cdot \left[ \frac{l_j}{l_{ij}} - 1 \right]$$  (1a)

where $l_j$, $l_{ij}$ and $A_j$ indicate the observable length, the unstretched length and the cross-sectional area of segment $j$, respectively and $c$ is the elasticity constant. In a
branched neurite each segment will be subjected to elastic tension and a tension equilibrium will exist. If we assume that the neurite is adhered to the substrate only at the growth cones and that the basis of the root segment, the cell body, is static, then at each branch point, where three segments are joined, the conditions:
\[
\sum_{j=1}^{3} f_j \cos \Theta_j = 0 \quad (1b)
\]
\[
\sum_{j=1}^{3} f_j \sin \Theta_j = 0 \quad (1c)
\]
will be fulfilled, where \( \Theta_j \) is the angle of segment \( j \) with respect to some frame of reference. The condition of a static cell body is fulfilled when the cell body assumes a static position, for example on laminin-coated substrates and when multiple neurites extend from the cell body (see section 2). Propagation of the growth cones will cause shifting of the branch points to meet the force equilibrium conditions [eqns (1b) and (1c)], resulting in adjusted observable lengths and orientations of the segments. From eqn (1a) it appears that the elasticity constant \( c \) cancels in eqns (1b) and (1c) and can be ignored. The positions of the branch points cannot be analytically inferred from the conditions. The numerical Newton–Raphson procedure (see Press et al., 1986) is used to find the equilibrium configuration for all branch points.

3.3. THE NEURON

The previous two subsections described the modeling of the outgrowth of a single neurite from the neuron. In most types of neurons, multiple neurites will grow out from the cell body simultaneously. During outgrowth the cell body generally attains a static position because (1) it is adhered to the substrate or (2) it is kept in a static position because of the force equilibrium of all outgrowing neurites. In both cases the single neurites will develop as if the cell body is firmly attached to the substrate. To study the shape of single neurites it is then sufficient to model only one neurite connected to a static cell body. Simulating the simultaneous outgrowth of several neurites emerging from one static cell body is then equivalent to simulating sequentially as many single neurites emerging from static cell bodies.

The orientations of neurites, i.e. the angles between their root segments, growing simultaneously from a cell body are not obtained in the sequential approach. Because our primary interest is in the shape of the neurite itself and the heavy computational burden of simultaneously simulating multiple neurites, we will proceed with the single neurite model to study neuritic shape.

3.4. ELONGATION

Experimental data on the elongation rate of neurites suggest a fairly constant rate (Bray, 1973; McCobb et al., 1988). In the model such a constant elongation rate of the observable length of a segment is assumed. The elongation rate of the unstretched length is calculated from this elongation rate by assuming that the tension in a
segment does not change during the propagation of growth cone $j$ at time step $t$ to $t+1$:

$$f_j^t = f_j^{t+1}. \tag{2a}$$

This implies two things. First, that the elongation rate of the unstretched length will be a function of the elastic tension, as put forward by Buxbaum & Heidemann (1988). Second, that the initial tension in the neurite, given at the start of growth, is the tension which the neurite would assume in time, its equilibrium value. The unstretched length at time $t+1$ can be calculated by substituting eqn (1a) at time $t$ and $t+1$ in eqn (2a):

$$l_j^{t+1} = \frac{l_j^{t+1}}{l_j^t}. \tag{2b}$$

At the start of growth the root segment is given an initial unstretched and an initial observable length and thus an initial tension. If we assume a constant elongation rate of the observable length, the elongation rate of the unstretched length will also be constant. This can be verified by substituting $l_j^{t+1} = l_j^t + \delta_j$, $\delta_j$ a constant and $l_j^{t+1} = l_j^t + \delta_j(t)$, $\delta_j(t)$ some function of $t$, in eqn (2b):

$$\frac{\delta_j}{\delta_j(t)} = \frac{l_j^t}{l_j^t} = \alpha(t). \tag{2c}$$

When eqn (2c) is compared to (1a) and (2a), it appears that $\alpha$ is constant because of the condition of equal forces as given in eqn (2a). To fulfill eqn (2c) with constant $\alpha$, $\delta_j(t)$ must be constant. Different segments will have different unstretched elongation rates when their stretching is not exactly equal. However, only minor differences in stretching will occur to fulfill the force equilibrium in the neuritic tree.

3.5. BRANCHING

When the angular distribution of the filopodia is strongly heterogeneous (e.g. bimodal) branching is likely to occur, as suggested by experimental results. As a measure of bimodality the variance of unit filopodial vector components perpendicular to their sum vector can be taken. The perpendicular variance $B$ is calculated by:

$$B = \frac{1}{n} \sum \sin^2 (\phi_i - \phi) \tag{3}$$

where $\phi_i$ is the angle of filopodium $i$ with a chosen frame of reference, $\phi$ = the angle of the sum vector of the filopodia with the same frame of reference, $i = 1, \ldots, n$, $n =$ total number of filopodia. $B$ will take values in $[0, 1]$. A low value of $B$ indicates a clustered distribution of the filopodial vectors around the mean direction, a high value indicates a bimodal distribution. Branching will occur when $B$ exceeds a given threshold $T$.

If $B$ exceeds $T$, the filopodia are split up in the two groups at both sides of the sum vector. Each group defines the direction of propagation for the daughter growth
OUTGROWTH OF BRANCHING NEURITES

cone at that side. For the relation of the parent segment radius \( r_0 \) to daughter segment radii \( r_{11}, r_{12} \) Rall (1959) proposed \( r_1^2 = r_{11}^2 + r_{12}^2 \), with \( e = 1.5 \). A corresponding relation will be assumed for the radii of the outgrowth cones: \( r_c^2 = r_{c1}^2 + r_{c2}^2 \), with \( e = 1.5 \), \( r_c \) the parent growth cone radius and \( r_{c1}, r_{c2} \) the daughter growth cone radii. Additionally we will assume that the ratio of the radii of the daughter growth cones is equal to the ratio of their number of filopodia:

\[
r_{c1}/r_{c2} = n_1/n_2
\]

where \( n_1, n_2 \) equal the number of filopodia of each of the daughters. In some cases the radii of parent and daughter growth cones were kept constant: \( r = r_{c1} = r_{c2} \).

3.6. ENVIRONMENT

Simple tissue cultures form a two-dimensional homogeneous substrate for developing neurites. Therefore we have assumed a two-dimensional substrate in the model, upon which identical adhesion sites are uniformly random dispersed. The density of adhesion sites is the only parameter describing the environment for the growth cones. The adhesion sites are dispersed at forehand and do not change position during the simulations.

We will also assume that the simulated neurite is not perceived by its own growth cones. Such an assumption will be valid for cells in which growth cones have a low probability of encountering their own neurite. This will be the case in young cells (Bray, 1973). With this assumption, the results of the model will apply best to these young cells.

4. Analysis of the Model

From the definition of the different parts of the model, properties concerning guidance and branching can be derived.

4.1. GUIDANCE

The direction of propagation is determined by the sum vector of the filopodial vectors, which depends completely on the location of the adhesion sites within the detection area. Because of the shape of the detection area, the filopodia do not appear on the back of the growth cone where the neurite is attached. Assuming a homogeneous surface with uniformly random spread adhesion sites, the distribution of the angles of the filopodia is uniform between \([-\frac{1}{2}\phi_o, \frac{1}{2}\phi_o]\), the angular width of the detection area. The expected direction \( E(\phi) = 0 \), which is forwards, in the direction of the symmetry axis of the detection area. Because of the randomness in number and position of the adhesion sites, the growth cone's direction of propagation will deviate from its expectation. Whenever there are more filopodia on one side of the growth cone, the growth cone will turn into that direction.
4.2. BRANCHING

In the analysis of the perpendicular variance $B$ it is assumed that the distribution of the adhesion sites on the surface is uniform, implying a uniform angular distribution of the filopodia. In order to evaluate the properties of the perpendicular variance $B$, the actual $\phi$ is simplified to its expectation $E(\phi) = 0$ and eqn (3) reduces to:

$$B^* = \frac{1}{n} \sum_i \sin^2(\phi_i).$$ (4a)

The expectation and variance of $B^*$ are:

$$E(B^*) = 0.5 \left[ 1 - \frac{\sin(\phi_o)}{\phi_o} \right]$$ (4b)

$$\text{var}(B^*) = \frac{1}{8n} \left\{ 1 + \frac{\sin(2\phi_o)}{2\phi_o} - 2 \left\{ \frac{\sin(\phi_o)}{\phi_o} \right\}^2 \right\}$$ (4c)

where $\phi_o$ is the opening angle of the detection area, while the number of filopodia $n$ is fixed.

When it is taken into account that $\phi$ will not be equal to its expectation of zero and $n$ is not fixed, it turns out that the expectation of $B$ depends on $n$, the number of filopodia. If $n \to \infty$ then var $(\phi) \to 0$, justifying the assumption $\phi = E(\phi)$. If $n = 0$, $B$ cannot be calculated, but is defined to be 0. If $n = 1$, $\phi_i = \phi$ and $B = 0$. If $n > 1$ the expectation of $B$ will increase with increasing $n$ until the value $E(B^*)$ is reached. Because the distribution of $B$ is constricted in $[0, 1]$, it will be skewed at low $n$.

![Graph](image)

**Fig. 2.** (●) Distribution of $B$ at 0.0158 sites $\mu m^{-2}$; (▲) distribution of $B$ at 0.063 sites $\mu m^{-2}$. Detection area radius: 11.61 $\mu m$, $\phi_o = \pi$. 
Figure 2 displays the distribution of \( B, P(\beta = B) \), for two densities of adhesion sites at a \( \phi_o \) of \( \pi \) radian. The distributions were generated by placing the detection area of the growth cone randomly in a homogeneous random environment of adhesion sites. Figure 2 shows that at the higher density the distribution is unimodal, with its mode at \( B = 0.5 \), as would be expected from (4b). At the lower density the distribution becomes bimodal, because of the high probability of detecting only 0 or 1 adhesion site within the detection area (resulting in \( B = 0 \)). The second mode is shifted from \( B = 0.5 \) towards \( B = 0.4 \). The variance has increased as expected from (4c).

In order to obtain a variance measure that is mainly sensitive to the distribution of the filopodia and less sensitive to the number of filopodia, the standardized \( B \) can be used

\[
B_s(n) = \frac{B - E(B(n))}{S(n)}
\]  

where \( E(B(n)) \) is the expectation and \( S \) is the standard deviation of \( B \) at \( n \) filopodia, \( n > 1 \). Because of the skewness of the distribution of \( B \) there will be a clear dependency of \( B_s \) on \( n \) when \( n \) is small. With \( n = 0 \), \( \text{var}(B) = 0 \) and the standardized version cannot be used.

The distribution of \( B \) can be given in terms of its two underlying distributions: the distribution \( P(N = n) \) of \( n \), the number of filopodia pointing to the detected adhesion sites, and the distribution \( P(\beta = B \mid N = n) \) of \( B \) given \( n \) filopodia. Because branching takes place whenever \( B \) exceeds \( T \), the distribution based on \( B \) of importance is:

\[
P(\beta > T \mid N = n) = \int_T^1 P(\beta = B \mid N = n) \, dB.
\]  

For a uniformly random distribution of adhesion sites \( P(N = n) \) will be a Poisson distribution with a mean of \( \tau = d r^2 \phi_o \) detected adhesion sites, where \( d = \) number of adhesion sites per unit area, \( r \) the growth cone radius and \( \phi_o r^2 \) the surface of the detection area. The distribution \( P(\beta > T) \) is given by

\[
P(\beta > T) = \sum_{n = 0}^{\infty} P(N = n) P(\beta > T \mid N = n).
\]  

The \( P(\beta > T) \) is the probability that a randomly placed growth cone detects a local environment in which it will branch.

The dependence of \( B \) on \( n \) is reflected in \( P(\beta > T) \). In growth cones with small \( n \), the term \( P(\beta > T \mid N \in \{0, 1\}) \equiv 0 \) will dominate the unconditional \( P(\beta > T) \), reducing the probability that such a growth cone will branch in a random selected environment. At large \( n \), the unstandardized version of \( B \) also yields a decreasing branching probability. \( B \) has a variance inversely proportional to \( n \) [eqn (4c)]. If \( T > E(B) \) then \( P(\beta > T) \to 0 \) as \( n \to \infty \), while \( P(\beta > T) \to 1 \) if \( T < E(B) \) and \( n \to \infty \). The standardized version \( B_s \) yields stabilizing \( P(\beta > T) \) for increasing \( n \). The effect of low \( n \) on \( P(\beta > T) \) is a general effect based on the assumption that small growth cones have
only a small number of filopodia and two or more filopodia are needed for branching. The effect of large \( n \) is highly dependent on whether or not \( B \) is standardized.

These effects of the size of the growth cone on \( P(\beta > T) \) translate directly to segment length. Because \( P(\beta > T) \) is constant for a certain segment, and the elongation rate is constant, the distribution of segment lengths will be exponential (Cox & Lewis, 1966) and the expected length is inversely proportional to \( P(\beta > T) \).

5. Analysis of the Model: Simulation

To study the development of a neurite as a whole, all the above mentioned processes were brought together in a simulation model.

5.1. PROCEDE

A simulation starts with a single growth cone on top of a segment with initial unstretched and observable length. During each simulation time step each growth cone samples the local environment, filopodial vectors to the adhesion sites are drawn and a decision on branching is made based on eqn (3). In case of propagation the growth cone moves in the direction of the sum filopodial vector and the unstretched length of the trailing segment is elongated. In case of branching, two daughter growth cones are formed according to section 3.4. After all growth cones of the neurite are

**Table 1**

(a) *Parameter estimations used in the simulations and their source*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>( v, ) growth rate of observable length</td>
<td>0.68 ( \mu m ) min(^{-1} )</td>
<td>Bray (1973)</td>
</tr>
<tr>
<td>( \phi_r, ) opening angle</td>
<td>( \pi ) radial</td>
<td>Estimated from filopodial distribution in Bray, 1973. Rounded afterwards.</td>
</tr>
<tr>
<td>( T, ) threshold</td>
<td>Unstandardized model: 0.6 or 0.8</td>
<td>No source</td>
</tr>
<tr>
<td></td>
<td>Standardized model: 1.0 or 1.5</td>
<td>No source</td>
</tr>
<tr>
<td>( d, ) adhesion sites per area</td>
<td>0.0315 ( \mu m^{-2} )</td>
<td>Resulting in a mean of ten or 20 filopodia for a starting growth cone.</td>
</tr>
<tr>
<td>( e, ) exponent in parent–daughter relation</td>
<td>0.0630 ( \mu m^{-2} )</td>
<td>Rall (1959)</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>

(b) *Initial values*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Initial value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r, ) cone radius</td>
<td>11.61 ( \mu m )</td>
<td>Bray &amp; Chapman (1985)</td>
</tr>
<tr>
<td>( l_r, ) unstretched length root segment</td>
<td>1 ( \mu m )</td>
<td>None</td>
</tr>
<tr>
<td>( l_o, ) observable length root segment</td>
<td>2 ( \mu m )</td>
<td>None</td>
</tr>
</tbody>
</table>
treated in this way, the neurite is brought into tension equilibrium by repositioning the branch points according to eqn (1).

Parameter estimations and initial values were as much as possible based on published data on tissue cultures [Tables 1(a) and (b)]. The total simulation time was estimated by means of the average length of unbranched root segments in Bray (1973): 180 µm. The growth rate of the neurites was 0·68 µm min⁻¹, indicating a growth time of about 268 min. Therefore the simulation step length was taken to be 2·5 min, resulting in a total simulation time of 250 min after 100 steps. The density of adhesion sites was estimated by the number of filopodia on a growth cone. The initial observable length was taken twice the initial unstretched length [Table 1(b)]. The elongation rate of the observable length is then twice the elongation rate of the unstretched length as shown in section 3.3. An example of a simulated tree is given in Fig. 3, together with the path followed by the growth cones.

Properties of the neuritic morphology generated by the model will be shown for a set of reference simulations of 100 independently simulated trees. The effect of changing parameter values and structural changes are studied in relation to the reference outcomes. The parameters varied were the threshold $T$ and the number of adhesion sites per unit area, $d$. The structural changes were the standardization of the perpendicular variance, the parent–daughter relation of growth cone radius and the application of the elastic updating as described in section 3.2. Each set of simulations consists

![Fig. 3. (a) Dendrites formed by a model simulation; (b) paths followed by the growth cones.](image-url)
of 100 independently simulated trees. The results are compared with respect to the centrifugal order–segment length relation, the intermediate angle distribution, topological asymmetry and the mean number of terminal segments of a tree.

The intermediate angle is the angle between the two daughter segments at a branch point (Uylings et al., 1986). The centrifugal ordering is a numbering system which defines the order of a segment as the number of branch points between the segment and the base of the tree. The topological asymmetry is derived from the partition asymmetry (van Pelt et al., 1989b):

\[ A(r, s) = \frac{|r - s|}{r + s - 2} \]

where \( r \) and \( s \) indicate the number of terminal segments of the two subtrees at a bifurcation. By definition we take \( A(1, 1) = 0 \). The topological asymmetry is the mean value of \( A(r, s) \), averaged over all branch points (van Pelt et al., 1989b; van Pelt et al., 1992).

5.2. RESULTS OF REFERENCE SIMULATION

The simple condition \( r_c = r_{c'} = r_z \) and the use of the unstandardized perpendicular variance \( B \) were chosen as a reference simulation. The threshold \( T \) was set to 0.6 and the density of adhesion sites was taken to be 0.0315 sites per \( \mu m^2 \).

The random positions of the adhesion sites in the environment, the only source of variance in the model, are reflected by the random fluctuations in the propagation direction of the simulated growth cone. In fact the movement of the growth cone is a directed random walk, because the detection area is only a part of a circle. The random walk is also restricted by the direction of the trailing segment, because the growth cone is in line with it. The longer the segment, the more it will prevent further deviations, and the deviation from the initial direction stabilizes. These aspects are clearly shown in Fig. 4, where the deviation from the initial direction stabilizes within 100 min of simulation time and the fluctuations around the mean direction of propagation become smaller with increasing time (and a longer trailing segment). Therefore, the simulated growth cones have a strong tendency to grow forwards, as observed in growing axons (Katz et al., 1984).

Two angular distributions have been obtained, the distribution of the intermediate angle at branching and the distribution of this angle in the eventual tree. The distribution in the eventual tree (Fig. 5) has a unimodal distribution with the mode at 0.675\( \pi \) radian. Compared with the angles at the time of branching (Fig. 5), it appears that the distribution is wider and the mode is slightly higher. The increase in variance is caused by the deviations of the direction of propagation of a growth cone from the initial direction (Fig. 4). However extreme deviations are avoided because, upon branching, the daughter growth cones are oriented to the largest amount of adhesion sites.

The distribution of the segment lengths at different orders, differentiated in terminal and intermediate segments, is displayed in Fig. 6(a). Terminal segments are longer
Fig. 4. Angular deviation from the initial direction of five modeled growth cones placed random in a homogeneous environment.

Fig. 5. (▲) Initial and final (●) angular distribution of dendrites generated in the reference simulation.
than intermediate segments of the same order, up to an order of about five. Segment length decreases with increasing order for both terminal and intermediate segments.

The outcomes for topological measures are given in Table 2. The topological asymmetry is 0.422, with a mean number of terminal segments of 45.50.

5.3. CHANGING THE THRESHOLD

Increasing the threshold from 0.6 to 0.8 will decrease $P(\beta > T)$. This results in a smaller number of terminal segments. Such a small number of terminal segments implies longer segment lengths. The strong decrease in mean number of terminal segments is illustrated by the outcomes of the simulations with $T=0.8$ (Table 2).
Table 2
Mean degree and topological asymmetry of model generated trees. Unstandardized perpendicular variance

<table>
<thead>
<tr>
<th>T=0.6</th>
<th>Mean degree (S.D.)</th>
<th>Asymmetry (S.D.)</th>
<th>T=0.8</th>
<th>Mean degree (S.D.)</th>
<th>Asymmetry (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>r_c = r_c1 = r_c2</td>
<td>45.50 (14.363)</td>
<td>0.422 (0.083)</td>
<td>1.07 (0.257)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>r_c' = r_c1 + r_c2</td>
<td>11.34 (2.829)</td>
<td>0.295 (0.123)</td>
<td>1.46 (1.660)</td>
<td>0.333 (0.255)</td>
<td></td>
</tr>
<tr>
<td>r_c = r_c1 = r_c2</td>
<td>21.43 (15.71)</td>
<td>0.442 (0.126)</td>
<td>1.01 (0.001)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>r_c' = r_c1 + r_c2</td>
<td>15.45 (5.474)</td>
<td>0.288 (0.108)</td>
<td>1.01 (0.001)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

e = 1.5; r_c = growth cone radius of parent segment; r_c1, r_c2 = ibid., daughter segments; d = number of adhesion sites per μm²; T = branch threshold.

5.4. CHANGING THE DENSITY OF ADHESION SITES

Increasing the density of adhesion sites from d=0.0315 to 0.0630 reduces the probability of branching as shown in section 4.2. As expected the mean number of terminal segments is reduced in the simulations (Table 2), but there was no effect on

![Fig. 7. Distributions of the intermediate angle for unstandardized simulations. Distribution based on 100 trees. (●) Reference; (■) decreasing growth cone radius; (▲) adhesion site density doubled; (◆) branch point fixed.](image)
the topological asymmetry and intermediate angles (compare Fig. 5 with Fig. 7). Terminal and intermediate segments become slightly longer [Fig. 6(b)].

5.5. CHANGING PARENT-DAUGHTER GROWTH CONE RADIUS RELATION

The change from equal growth cone radii to a parent-daughter relation of \( r_{c1}^{1.5} = r_{c1}^{1.5} + r_{c2}^{1.5} (e = 1.5) \) implements a decrement in growth cone radius after branching. From the formulation of the branching probability it is expected that this probability decreases with smaller growth cone radius, thus yielding longer segments [Fig. 6(c)]. With intermediate segments, the order–segment length relation shows increasing lengths with increasing order, in contrast with trees having a constant radius. As expected from the longer intermediate segments, terminal segments decrease faster in length compared with trees with constant growth cone radius.

![Fig. 8. Centrifugal order-segment length relation in the standardized simulations. Mean values based on simulation of 100 trees. (a) Reference simulation; (b) simulation with increased number of effective adhesion sites; (c) simulation with decreasing radius after branching; (d) simulation with branch threshold increased by 150%. (■) Intermediate segments; (●) terminal segments.](image)
The distribution of the intermediate angle (Fig. 7) hardly changes. The topological asymmetry becomes smaller, indicating that more symmetric trees are formed.

5.6. OMISSION OF ELASTIC UPDATE

When the elastic update is omitted and the positions of the branch points are fixed, the mean number of terminal segments becomes 53.8 (S.D.: 1.74). This number reaches the maximum number of terminal segments (being 50) which is allowed during simulation because of computer memory restrictions. The actual mean number of terminal segments is more than the maximum number of terminal segments because of simultaneous branch events in the last simulation step. The simulation is terminated when this maximum is reached, and the simulation time becomes shorter. This is reflected in the segment length–order relation [Fig. 6(d)], which has the appearance of the reference simulation but the lengths are smaller. The angular distribution resembles the reference, but the mode is 0.1 radian smaller and the variance has increased (Fig. 7). The topological asymmetry is about the same as the reference simulation, 0.433 (S.D.: 0.054).

5.7. STANDARDIZED PERPENDICULAR VARIANCE

In most of the cases the conclusions reached when the unstandardized perpendicular variance $B$ is used also hold when the standardized perpendicular variance $B_s$ is
**Table 3**

Mean degree and topological asymmetry of model generated trees. Standardized perpendicular variance

<table>
<thead>
<tr>
<th></th>
<th>Mean degree (S.D.)</th>
<th>Asymmetry (S.D.)</th>
<th>Mean degree (S.D.)</th>
<th>Asymmetry (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T=1-0$</td>
<td></td>
<td>$T=1-5$</td>
<td></td>
</tr>
<tr>
<td>$r_e = r_{e1} = r_{e2}$</td>
<td>23.42 (17.007)</td>
<td>0.429 (0.118)</td>
<td>3.01 (2.359)</td>
<td>0.369 (0.279)</td>
</tr>
<tr>
<td>$r_e^* = r_{e1}^* + r_{e2}^*$</td>
<td>7.17 (2.648)</td>
<td>0.316 (0.167)</td>
<td>2.96 (2.020)</td>
<td>0.293 (0.253)</td>
</tr>
<tr>
<td>$r_e = r_{e1} = r_{e2}$</td>
<td>34.63 (18.525)</td>
<td>0.444 (0.094)</td>
<td>4.37 (3.110)</td>
<td>0.337 (0.260)</td>
</tr>
<tr>
<td>$r_e^* = r_{e1}^* + r_{e2}^*$</td>
<td>10.13 (4.153)</td>
<td>0.297 (0.158)</td>
<td>3.62 (2.386)</td>
<td>0.330 (0.264)</td>
</tr>
</tbody>
</table>

$d = 0.0315$

dr = $r_{e1} = r_{e2}$

dr = $r_{e1}^* + r_{e2}^*$

e = 1.5; $r_e =$ growth cone radius of parent segment; $r_{e1}, r_{e2} =$ *ibid.*, daughter segments; $d =$ number of adhesion sites per $\mu m^2$; $T =$ branch threshold.

used (Figs 8 and 9 and Table 3). In contrast to the simulations with an understandardized perpendicular variance, the mean number of terminal segments increases with an increasing number of adhesion sites.

**6. Comparison with Tissue-Cultured Neurites**

Bray (1973) describes 23 neurites from ten cells originating from the cervical ganglion of rat, grown in tissue culture without any treatments. From the published figures of branching neurites the topological asymmetry, the number of terminal segments, the length distribution and the distribution of the intermediate angle were measured.

The metrical distributions of Bray’s neurites show that terminal segments are longer than intermediate segments of the same order (Fig. 10). Terminal segments decrease in length with increasing order. The same result is reached with the simulated neurites (Figs 6 and 8), even the length of the segments is in the same order of magnitude. In the simulated trees however, constant lengths of intermediate segments are not observed. Rather, trees with a constant growth cone radius show a decreasing intermediate segment length, while the trees with decreasing growth cone radius show an increasing intermediate segment length with increasing order. A smaller decrease in growth cone radius may well yield constant intermediate segment lengths.

Comparison with the angular distributions generated by different simulations (Figs 5, 7 and 9) shows that the cultured neurites have a distribution with a larger variance and a somewhat lower mean (Fig. 11). However, observed and simulated distributions are in the same range, with their modes around 0.6 $\pi$ radian.

The neurites of Bray have a topological asymmetry of 0.306 (S.D.: 0.269), and a mean number of terminal segments of 4.25 (S.D.: 2.954). The mean number of
Fig. 10. Centrifugal order-segment length relation of figures of neurites published by Bray (1973). (▲) Intermediate segments; (●) terminal segments.

Fig. 11. Distribution of intermediate angles in figures of neurites published by Bray (1973).
terminal segments of simulated trees with constant growth cone radius is higher (Tables 2 and 3). Most of the trees with decreasing growth cone radius have asymmetries close to the experimental 0.306, in good agreement with the experimental data. Although the mean number of terminal segments of these trees is much lower than in trees with constant growth cone radius, it is still two- to three-fold the mean number of tissue-cultured trees (Tables 2 and 3).

7. Discussion

The model of neuritic outgrowth presented here is based mainly on processes at the level of the growth cone. The neurite evolves because the propagating growth cones are on top of elastic segments which they elongate. Assuming an environment with uniformly random dispersed adhesion sites, the present model is able to reproduce topological and metrical characteristics of untreated neurons from the cervical ganglia of the rat. Modeled trees with a decreasing growth cone radius agree better with the experimental trees than do modeled trees with constant growth cone radius. This is most evident for the topological asymmetry and suggests that the growth cone radius indeed diminishes after branching and the number of filopodia diminishes with order.

However, a fast decrease of growth cone radius implies a rapid increase in the probability that a cone has 0 or 1 filopodia. In tissue cultures growth cones with 0 or 1 filopodia are seldom observed, so it may not be realistic to have any cones with so few filopodia. This would suggest that growth cones do decrease in radius with increasing order, but not as rapid as $e = 1.5$. Data on parent–daughter segment radii suggest a relation with $e = 1.5$ or a somewhat higher $e$ for segments (Kerneli & Zwaagstra, 1989), and it may well be that growth cones decrease more slowly in radius than segments.

As in cultured neurites (Bray, 1973) and in vivo neurites (Parnavelas & Uylings, 1980; Uylings et al., 1989a), the simulated trees have longer terminal segments as compared with intermediate segments of the same order. Terminal segments decrease in length at higher order. Starting with a set of growth cones, some of them will be branched after a certain time. The intermediate segments created by the branching of these growth cones will be shorter than the trailing, terminal, segments of the still unbranched growth cones, only because they have branched and thus stopped growing. The same argument will do for the decreasing segment length at higher order. Growth cones at a high order have less time to grow than growth cones at a lower order, which started earlier with growing in their particular order. The consequences of the constant elongation rate at the tip and a constant branch probability will be studied analytically in a following paper.

A similarity was not only found in topological and metrical characteristics, but also in outgrowth dynamics. Due to the limited number of filopodia which steer the growth cone, fluctuations from the mean path were observed during simulated outgrowth. Such fluctuations around a mean path have also been described for outgrowing axons (Katz et al., 1984; Katz, 1985), suggesting that also in outgrowing axons the fluctuating path of the growth cone may be due to a limited number of
"steering structures". These structures may well be the filopodia, which would support the basic mechanism for guidance and branching in the model.

Two changes in the model had a drastic effect on the outcomes: increasing threshold $T$ and introducing a decreasing growth cone radius after branching. Both cause a decrease of the branch probability as defined in section 4.2, resulting in longer segments and a lower number of terminal segments. In accordance with topological models with a decreasing branch probability at higher order (van Pelt & Verwer, 1986), simulated trees with decreasing radius are more symmetrical than are trees with a constant radius. These results pinpoint on the branch probability as a major determinant of morphology in the model.

With respect to axonal guidance other models have been developed, which do not include branching and do not explicitly formulate the elongation rate. Gierer (1981) and Bonhoeffer & Gierer (1984) present a model in which axonal guidance is due to amplification of environmental gradients in the growth cone. The fairly accurate way in which axons find their targets within the optic tectum is explained in this way. Katz & Lasek (1985) present a model for guidance in sheets of tissue. Guidance is achieved by a difference in adhesion between the other axons and the surface on which they are growing and by highly adhesive pathways. The growth cones are restricted in the maximum angle they can turn during a time step.

The model presented here is built upon the same underlying assumptions as these two models: the growth cone is the structure which samples the environment and environmental heterogeneities guide the growth cone. All three models ascribe a dominant influence to the environment in guiding the growth cone. Because the present model was set up for a homogeneous environment, no attention has been paid to the possibility of different types of adhesion sites. However, they are easily included in the model by the introduction of weight factors in the calculation of the sum vector and the perpendicular variance $B$. Each type of adhesion site will have its own weight factor. Weight factors could further be changed by the presence of modulating chemical compounds or electric fields, accounting for guidance by such compounds.

Opinions differ to the role of the growth cone in neuritic elongation. Bray (1987) argues that the pulling growth cone is the main factor in elongation, the "pull mode" of growth. In contrast, Goldberg & Burmeister (1988, 1989) argue that at the growth cone material is inserted and this addition of material is the main factor in outgrowth, the "push mode" of growth. The assumptions in the model refer to an inbetween situation. Segments grow by addition of material into an elastic segment. The pulling growth cone causes stretching of the segment. The overall elongation is thus caused by both a push and a pull mechanism. Exactly this mode of growth is shown by Lamoureux et al. (1989) for chick sensory axons.

In conclusion, the present model supports the idea that indeed characteristics in the geometry of neuritic trees arise from mechanisms acting at the level of the growth cone. The variability in the environment, as single source of variance, causes a whole range of different neurites, although the rules by which each neurite grows are the same. It is the interaction between the environment and the growth rules of the neurite which determines the eventual morphology of the neurite.
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REFERENCES