Competition in Neuronal Morphogenesis and the Development of Nerve Connections

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ABSTRACT

During the development of the nervous system, neurons form their characteristic morphologies and become assembled into synaptically connected networks. In many of the developmental phases that can be distinguished, e.g., axonal differentiation, neurite elongation and branching, and synapse rearrangement, competition plays an important role. Focusing on competition, we review model studies on neuronal morphogenesis and the development of nerve connections.

11.1. INTRODUCTION

During development, neurons become assembled into functional networks by growing out axons and dendrites (collectively called neurites), which connect synaptically to other neurons. A number of developmental phases can be distinguished.

Early dendritic and axonal morphogenesis. The neurons begin to grow by projecting many broad, sheet-like extensions, called lamellipodia, which subsequently condense into a number of small undifferentiated neurites of approximately equal length (1). Eventually, one of the neurites (usually the longest) increases its growth rate—while at the same time the growth rate of the remaining neurites is reduced—and differentiates into an axon. The remaining neurites become later differentiated as dendrites and form characteristic branching patterns. The development of dendritic morphology proceeds by way of the dynamic behavior of growth cones, which are specialized structures at the terminal ends of outgrowing neurites and which mediate neurite elongation and branching (2). Among the many intra- and extracellular mechanisms involved in growth cone behavior are intracellular calcium levels, signal transduction cascades and cytoskeletal changes (3).

Axon guidance and synapse formation. The axons need to migrate to their targets, and one of the mechanisms by which this is achieved is by the diffusion of chemoattractant molecules from the target through the extracellular space (4). This creates a gradient of increasing concentration, which the growth cone at the tip of a migrating axon can sense and follow (5). Axons are also repelled by diffusible molecules that are secreted by tissues the axons need to grow away from. In addition
to diffusible molecules, axons are also attracted and repelled by surface-bound molecules on other cells, and in the extracellular matrix (4). Once the axons have arrived at their targets, they form synaptic connections by transforming their growth cones into synapses.

**Synapse rearrangement.** The phase of synapse formation is followed by a phase of refinement, including both the formation of new synapses and the elimination of existing synapses (6, 7). This process often involves withdrawal of some axons and, thus, a reduction in the number of axons innervating an individual target cell. In some cases, withdrawal of axons continues until the target is innervated by just a single axon, whereas in most other cases several innervating axons remain (8–10) (see also Subheading 11.4.).

Competition plays an important role in many of the above described developmental phases. In axonal differentiation, all the neurites have the potential to develop into the axon (11, 12). In experiments in which the axon is transected at various distances from the soma, the longest neurite remaining after transection usually becomes the axon, regardless of whether it was previously an axon or a dendrite (12). Thus, axonal differentiation appears to be a competitive process in which the growth rate of the longest neurite is accelerated at the expense of all the other neurites, whose growth become inhibited (1, 13) (see also 14).

Some form of competition is also operative in the formation of dendritic trees: the branching probability of an individual growth cone (i.e., terminal segment) appears to decrease with the number of other growth cones in the tree. Such a dependence turns out to be necessary for reducing the proliferating effect of the increasing number of growth cones (see Subheading 11.2.). Competitive effects between neurites are expected to occur also in the elongation of neurites: the proteins upon which elongation depend (namely, tubulin and microtubule-associated proteins) are produced in the soma and need to be divided among all the growing neurites of a neuron (15). Competition for tubulin could explain the observation that sometimes only one of the daughter growth cones propagate after branching, while the other stays dormant for a long time (see Subheading 11.3.1.).

Competition between innervating axons for target-derived neurotrophic factors is thought to be involved in the withdrawal of axons (10, 16). The cells that act as targets for the innervating axons release limited amounts of neurotrophic factors, which are taken up by the axons via specific receptors at their terminals and which affect the growth and branching of the axons (17, 18).

To gain a real understanding of nervous system development and function, experimental work needs to be complemented by theoretical analysis and computer simulation. Even for biological systems in which all the components are known, computational models are necessary to explore and understand how the components interact to make the system work and how phenomena at different levels of organization or description are linked. In this chapter, we discuss (i) models of the development of dendritic morphology, focusing on competitive phenomena (Subheadings 11.2. and 11.3.); and (ii) models of competition between innervating axons in the refinement of connections (subheading 11.4.). For a model on the role of competition in axonal differentiation, see (14). For models of axon guidance and fasciculation, see (19).
For all models we present, the various components of the model are relatively
uncontroversial biologically, and the aim is to explore and understand quantitatively
the consequences of the interactions between these components, in terms of the
phenomena and data the model can generate. This provides hypotheses and predictions
about these phenomena and data at a lower level of organization or description. For
example, neuronal morphology is linked to the actions of growth cones (Subheading
11.2.); the phenomenon of dormant growth cones is linked to competition, at the
molecular level, for tubulin (Subheading 11.3.1.); and axonal competition is linked to
the actions and biochemistry of neurotrophins (Subheading 11.4.2.).

11.2. DEVELOPMENT OF DENDRITIC MORPHOLOGY:
A STOCHASTIC MODEL

Dendritic branching patterns emerge from a developmental process of neurite elon-
gation and branching. This process is mediated by growth cones, which, under the
influence of intracellular and extracellular mechanisms, show highly dynamic behav-
ior, such as advance, reorientation, splitting, shape and speed changes, retraction, and
even complete disappearance. Outgrowth is, therefore, not a regular process of con-
tinued elongation and branching; nevertheless, it eventually results in dendritic branching
patterns that are typical for the type of neurons under consideration.

Modeling dendritic branching patterns from a developmental point of view raises
the question at which level of detail the growth process should be described. In our
modeling approach, we assume that at a sufficiently coarse time scale the averaged
outcome of all the underlying growth processes can be described as a sustained sto-
chastic process of elongation and branching. The stochasticity assumption is warranted
because of the multitude of mechanisms that determine the behavior of growth cones.

In our dendritic growth model (recently reviewed in [20,21]), a distinction is made
between topological and metrical properties of dendritic trees. Topological properties
emerge from the branching process as segments increase in number and develop a
particular connectivity pattern. Metrical properties emerge from both the branching
and the elongation process. The dendritic growth model has first been developed and
validated for the branching process. Later, elongation was included; this has the advan-
tage that the optimization of the metrical properties can be built upon an already opti-
mized branching process.

Modeling the dendritic branching process. To describe the branching process, the
total developmental period $T$ is divided into a series of $N$ time bins. In each time bin $i$,
a terminal segment (growth cone) may branch with a probability given by:

$$p_i(n_i, \gamma) = D n_i^{-E} \times 2^{-S_i} C_i$$  \[Eq. 1\]

Parameter $D$ determines the basic branching probability, which is taken equal to
$D = B/N$, with $B$ denoting the expected number of branching events of an isolated segment
during the full period $T$. With the term $n_i^{-E}$, the branching probability is made depen-
dent on the number $n_i$ of terminal segments (this may be thought of as representing
some form of competition; see below). The strength of this dependence is determined
by parameter $E$. With the term $2^{-S_i} C_i$, the branching probability is made dependent on
the centrifugal order $\gamma$ of the terminal segment (see Fig. 1), thus allowing a modulation
of the branching probabilities over the different terminal segments in the dendritic tree. The strength of this modulation is determined by parameter $S$. The normalization constant $C_i = n_i / \sum_{j=1}^{n_i} 2^{-S_j}$ ensures that this modulation does not change the mean branching probability $Dn_i^E$, averaged over all terminal segments in the tree. Thus, the term controls the rate of increase of the number of segments, while the topological structure is under control of the modulation $2^{-S_i/C_i}$. The number $N$ of time bins can be chosen arbitrarily, but such that the branching probability per time bin remains much smaller than one, thus making the probability of more than one branching event per time bin negligibly small.

During outgrowth, an increasing number of terminal segments is participating in the branching process, and this proliferation strongly determines the rate with which the number of terminal segments increases. In the model, this proliferation is kept under control by making the branching probability dependent on the total number of segments, via parameter $E$. Figure 2A illustrates how fast the number of terminal segments increases for the unrestricted case $E = 0$, i.e., when the branching probability per time bin remains constant (Fig. 2D). For $E = 1$, in contrast, the branching probability is inversely proportional to the total number of terminal segments in the tree (Fig. 2F), resulting in a linearly increasing number of branch points up to the value of parameter $B$ (Fig. 2C). (Note that a binary tree with 3 branch points has 4 terminal segments.) The branching parameters $B$ and $E$ determine the growth rate not only of the mean number of terminal segments but also of the standard deviation of the terminal segment number distribution, as is shown in Figure 2. This is expressed also in the shape of this distribution, with smaller means and standard deviations (SDs) for increasing values of $E$ (Fig. 3). Figure 4 shows how the mean and SD depend on the parameters $B$ and $E$. For a given value of $B$, the mean number of terminal segments decreases with increasing
Fig. 2. (A–C) Growth curves of the mean and the SD of the number of terminal segments and (D–F) the course of the branching probability of a terminal segment per time bin, plotted vs a time bin scale with 500 time bins. The curves are calculated for $B = 3$, and for three values of $E$ with $E = 0$ (A,D), $E = 0.5$ (B,E), and $E = 1$ (C,F). The figures illustrate how parameter $E$ influences the growth curves and the course of the branching probability. Panels (G–L) are obtained by a nonlinear (exponential with exponent 3) mapping of the time bin scale with 500 time bins onto a continuous time scale with an arbitrary duration of 3 wk. Panels (J–L) display the time course of the branching probability of a terminal segment per hour. These panels illustrate that the shape of the growth curves is changed by the mapping but that the relation between mean and SD is maintained.
Fig. 3. Frequency distributions of the number of terminal segments per dendritic tree as produced for $B = 3$ and different values of the branching parameter $E$.

Fig. 4. Map of the number of terminal segments per dendritic tree vs the branching parameter $E$, for different values of the branching parameter $B$. The figure shows how the number of terminal segments, for a given $B$, decreases with increasing values of $E$. The figure also shows that a given number of terminal segments (say 10) can be produced by different combinations of parameters $B$ and $E$, but that for these combinations the standard deviation decreases for increasing values of $E$. Finally, the figure shows that for $E = 1$, the mean number of branch points in a tree equals the branching parameter $B$. Note that for binary trees the number of terminal segments is equal to the number of branch points plus one.
values of $E$. This reducing effect of parameter $E$ on the growth of the total number of terminal segments may represent some kind of competition between growth cones for branching. Trees of a given size can be produced by many combinations of the parameters $B$ and $E$, where higher values of $E$, resulting in a lower growth rate, should be accompanied by higher values of $B$. However, higher $E$ values also result in lower values of the SD, as indicated in Figure 4, where the horizontal line at, for instance, degree 10 crosses (dotted) curves of lower SD value for increasing $E$. Matching to observed SD values finally determines which $E$ and $B$ values most optimally predict the observed mean and SD values. The optimized $B$ and $E$ values tend to show clustering for different cell types, as was shown in (22) and is illustrated in Figure 5. Especially the pyramidal cell group shows significantly higher $E$ values than the other cell groups, suggesting that these dendrites develop under stronger competitive conditions. The clustering suggests also a differentiation in $B$ values between the other cell groups. Although the statistics are still poor, one may conclude from these findings that the branching parameters $B$ and $E$ indeed represent cell type-specific characteristics of dendritic branching patterns.
Modeling the dendritic branching process in continuous time. To describe the branching process in continuous time, the time bin scale needs to be mapped onto a real time scale. The equation for the branching probability per time bin then transforms into a branching probability per unit of time:

$$p_i(n_t, \gamma) = D(t)n_t^{-\kappa}2^{-Si}C_i$$

[Eq. 2]

with parameter $D(t)$ denoting the basic branching rate per unit of time. Time bins will obtain equal durations in a linear mapping, but may have different durations in a nonlinear mapping. An example is given in Figure 2G–L, which illustrates how the growth curves and the probability curves change when the time bin scale (with 500 bins) is exponentially mapped onto a continuous time scale of 504 h (3 wk). Note that the relation between mean and SD is maintained, being independent of the type of mapping.

Modeling the elongation process. Once the branching process has been optimized to the observed data set, the metrical properties can be modeled. To this end, newly formed daughter segments at a branching event are given an initial length and an elongation rate for the period of time up to the moment they branch again. Both the initial lengths and the elongation rates are randomly drawn from gamma distributions, with mean and SD values of $\bar{l}_m$, $\sigma_{ln}$, $\bar{v}$, and $\sigma_v$, respectively.

Results of the dendritic growth model. The model has been applied to dendritic data sets of a variety of cell types, including rat large layer 5 pyramidal neurons (20), small layer 5 pyramidal neurons (23), layer 2/3 pyramidal neurons (21), guinea pig cerebellar Purkinje cells (21), cat deep layer superior colliculus neurons (24), and rat cortical multipolar nonpyramidal neurons (25). In all these examples, the dendritic shape properties were well approximated up to the very details of their distributions.

11.3. NEURITE ELONGATION AND BRANCHING: CELL BIOLOGICAL MECHANISMS

Most models of the development of dendritic morphology describe neurite elongation and branching in a stochastic manner. Although these models are very successful at generating the observed variation in dendritic branching patterns (see Subheading 11.2.), they do not clarify how the biological mechanisms underlying neurite outgrowth are involved, namely, the dynamics of the tubulin and actin cytoskeleton. In this section, we present models that study the role of tubulin dynamics in neurite outgrowth.

11.3.1. Neurite Elongation as a Result of Tubulin Polymerization

The length of a neurite is determined by its microtubules, which are long polymers of tubulin present throughout the entire neurite. Tubulin monomers are produced in the cell body and are transported down the neurite to the growth cone. Polymerization of tubulin, which occurs mainly in the growth cone, elongates the microtubules and thus the neurite. The rates of tubulin assembly and disassembly are influenced by the actin cytoskeleton in the growth cone, by microtubule-associated proteins (MAPs), and by (activity-dependent) changes in the intracellular calcium concentration (26–30).

In (31) and (15), the consequences of the interactions between tubulin transport and tubulin (dis)assembly are explored. The model in (31) is based on the model in (15) and describes neurite elongation and retraction as the result of tubulin assembly and disas-
assembly. A simple compartmental model of a single neuron with $n$ different neurites is considered (Fig. 6). There is one compartment for the cell body and one compartment for the growth cone of each neurite $i$ ($i = 1, \ldots, n$). The time-dependent changes of the neurite length $L_i$, the concentration $C_0$ of tubulin in the cell body, and the concentrations $C_i$ of tubulin in the growth cones are modeled. Tubulin is produced in the cell body, at rate $s$, and is transported into the growth cones of the different neurites by diffusion and active transport, with diffusion constant $D$ and rate constant $f$, respectively. At the growth cones, concentration-dependent assembly of tubulin into microtubules takes place, which elongates the trailing neurite. Disassembly of microtubules into tubulin causes the neurite to retract. The rate constants $a_i$ and $b_i$, for, respectively, assembly and disassembly are taken slightly different in different neurites. Differences in rate constants between neurites can arise as a result of differences between neurites in electrical activity (which affects the concentration of intracellular calcium), in the actin cytoskeleton of the growth cones, or in the state or concentration of MAPs. Finally, tubulin is also subjected to degradation, with rate constant $g$, both in the cell body and in the growth cone. Thus, the rates of change of $L_i$, $C_i$, and $C_0$ become:

$$\frac{dL_i}{dt} = a_iC_i - b_i$$  \hspace{1cm} [Eq. 3]

$$\frac{dC_i}{dt} = b_i - a_iC_i + \frac{D}{L_i + k} (C_0 - C_i) + fC_0 - gC_i$$  \hspace{1cm} [Eq. 4]

$$\frac{dC_0}{dt} = s - \sum_{i=1}^{n} \frac{D}{L_i + k} (C_0 - C_i) - \sum_{i=1}^{n} fC_0 - gC_0$$  \hspace{1cm} [Eq. 5]
Fig. 7. Results of the compartmental model of a single neuron with two neurites. Neurite 1 has a higher rate constant for tubulin assembly. As a result, neurite 1 can slow down (A) or even prevent (B) the growth of the other neurite. Stopping the growth of neurite 1 triggers, after a time delay, the growth of the other neurite (C). Parameters (all units arbitrary): $b_1 = b_2 = 0.01$, $D = 0.5$, $g = 0.1$, $s = 0.07$, $f = 0$, and $k = 1$. In (A), $a_1 = 0.09$ and $a_2 = 0.06$. In (B) and (C), $a_1 = 0.3$ and $a_2 = 0.05$. From (31) with permission.

where $k$ is the distance between the centres of the cell body and growth cone compartment when $L_z = 0$. In (15), there is no degradation of tubulin, which is biologically not plausible and which makes the mathematical analysis more difficult, and no active transport of tubulin.

The analysis of the model shows that small differences between neurites in their rate constants for assembly and/or disassembly (e.g., as a result of differences between neurites in intracellular calcium concentration) lead to competition between growing neurites of the same neuron (also reported in (15)). This competition emerges as a result of the interactions between tubulin-mediated neurite elongation and transport of tubulin. If one of the neurites has a higher rate constant for tubulin assembly and/or a lower rate of disassembly, it can slow down (Fig. 7A) or even prevent (Fig. 7B) the outgrowth of the other neurites for a considerable period of time (i.e., they are “dormant”), by using up all the tubulin produced in the soma. Only after the fastest growing neurite has reached a certain length (the longer the neurite, the smaller the amount of tubulin that is transported by diffusion per unit time) can the tubulin concentration in the growth cones of the other neurites increase, causing them to grow out. The smaller the rate of production of tubulin in the cell body, the bigger this period of dormancy.

In (31), it was shown that stopping the outgrowth of the fastest growing neurite (e.g., representing the physiological situation that a neurite has reached its target) can “awaken” the dormant growth cones, which then, after a characteristic delay, start growing out (Fig. 7C). The length of the delay is determined by the time it takes for the tubulin concentration to build up to the value where the rate of assembly ($a_iC_i$) is bigger than the rate of disassembly ($b_j$).

Preliminary results show that the higher the relative contribution of the active component (parameter $f$) to the transport process, the smaller the competitive effects. In more detailed compartmental models (32), in which each neurite is divided into many compartments, we found very similar results as those reported here.
The model can account for the occurrence of "dormant growth cones" (33)—the observation that, after branching, only one of the daughter growth cones propagates. The prediction of the model that there should be competition between growing neurites of the same neuron has recently been confirmed experimentally (G.J.A. Ramakers, unpublished results). These findings show that (i) when one neurite stops growing out, other neurites (after a certain delay, as in the model) start growing out; and (ii) when more neurites are growing out at the same time, the rate of outgrowth is smaller than when only a single neurite is growing out. To test whether this is indeed due to competition for tubulin, as our model suggests, the concentration of tubulin in growth cones should be monitored during outgrowth. The model predicts that the concentration of tubulin in growth cones that are not growing out should be below the critical value [the concentration of tubulin at which assembly \((a_C)\) just equals disassembly \((b_d)\)].

11.3.2. The Role of Microtubule-Associated Proteins in Neurite Elongation and Branching

The tubulin dynamics is influenced by many modulators, among which the MAPs play a prominent role (34). They regulate not only assembly and disassembly, but also the bundling and spacing of microtubules. The phosphorylation state of MAPs affects their function (28,29). When MAPs are dephosphorylated, they promote tubulin assembly and microtubule bundling and so promote neurite elongation (35). When MAPs are phosphorylated, they inhibit assembly and bundling; the spacing between microtubule bundles increases, which favors dendritic branching (36). It has been proposed that the rates of elongation and branching are determined by the relative concentrations of phosphorylated and dephosphorylated MAPs (37,38). This is itself dependent on the concentration of intracellular calcium, which regulate both phosphorylation and dephosphorylation through the actions of calmodulin-dependent protein kinase 2 and calcineurin (28,29,39,40). Using a compartmental model for elongation and branching, Hely et al. (38) studied what the implications are of the interactions between the calcium dynamics (influx of calcium along, and diffusion within, the whole dendritic tree) and the effects of calcium on MAP (de)phosphorylation. In the model, the ratio of the concentrations of phosphorylated and dephosphorylated MAPs at the tip of a terminal segment (i.e., the growth cone) determines the branching probability and the rate of elongation. MAPs are produced in the soma and are transported to the growth cone by diffusion and active transport. One sigmoidal function is used to describe how the rate of MAP phosphorylation depends on the concentration of calcium in the growth cone; another sigmoidal function is used to describe how dephosphorylation depends on calcium. One result of the model is that the relative position of these two functions, together with the calcium dynamics, determines what dendritic structure will develop. As the tree grows, the calcium concentration in the terminal segments increases. The concentration is highest in the terminal segments and lowest in the soma (because of the higher surface-to-volume ratio in the thin terminal segments). As the tree grows, the terminal segments become farther away from the soma, which acts as a sink for calcium, so that the calcium concentration in the terminal segments increases. Depending on the relative position of the two sigmoidal functions, this increased calcium concentration leads either to a lower branching probability (producing trees in which the
terminal segments are longer than the proximal segments, e.g., as in the basal dendrites of pyramidal neurons [41]) or to a lower branching probability (producing trees in which the terminal segments are shorter than the proximal segments, e.g., as in cultured hippocampal neurons [42]). Thus, given a particular branching pattern, the model predicts how the functions relating calcium with phosphorylation and dephosphorylation should be in order to produce this.

11.4. COMPETITION BETWEEN AXONS IN THE REFINEMENT OF NEURAL CIRCUITS

During development, the refinement of neural circuits involves both the formation of new connections and the elimination of existing connections (6, 7). Neurons, and other cell types, often are initially innervated by more axons than ultimately maintain into adulthood (7, 43). This initial hyperinnervation followed by elimination occurs, for example, in the development of connections between motor neurons and muscle fibers (8, 9), where elimination of axons continues until each muscle fiber is innervated by just a single axon, and in the formation of ocular dominance columns in the visual cortex (44, 43). Although there is a reduction in the number of axons that an individual target receives, the total number of synapses onto a target often increases (both in the visual and in the neuromuscular system), because of further arborization of the remaining axons (46–48).

The process that reduces the number of axons innervating a postsynaptic cell is often referred to as axonal or synaptic competition. In particular, it is believed that axons compete for neurotrophic factors, survival- or growth-promoting substances, released by the postsynaptic cells upon which the axons innervate (10, 16). During an earlier stage of development, when initial synaptic contacts are made, these neurotrophic factors have a well-established role in the regulation of neuronal survival (49, 50). But many studies now indicate that neurotrophic factors may also be involved in the later stages of development, when there is further growth and elimination of innervation (for a critical review, see [51]). For example, neurotrophic factors have been shown to regulate the degree of arborization of axons (e.g., see [52]; for more references, see Subheading 11.4.2.).

Although the notion of competition is commonly used in neurobiology, the process is not well understood, and only a few formal models exist (for an extensive review, see [48]).

11.4.1. Competition Through Synaptic Normalization and Modified Hebbian Learning Rules

Most computational models of the development of nerve connections, especially models of the formation of ocular dominance columns, typically enforce competition rather than model its putative underlying mechanisms explicitly (for a review, see [53]). To see how competition between input connections can be enforced, consider \( n \) inputs, with synaptic strengths \( w_i(t) \) \((i = 1, \ldots, n)\), impinging on a given postsynaptic cell at time \( t \). Simple Hebbian rules for the change \( \Delta w_i(t) \) in synaptic strength in time interval \( \Delta t \) state that the synaptic strength should grow in proportion to the product of the postsynaptic activity level \( y(t) \) and the presynaptic activity level \( x_i(t) \) of the \( i \)th input. Thus:
\[ \Delta w_{i}(t) \propto y(t)x_{i}(t)\Delta t \]  
[Eq. 6]

If two inputs (e.g., two eyes) innervate a common target and if the activity level in both inputs is sufficient to achieve potentiation, then this rule causes both pathways to be strongly potentiated, and no segregation (ocular dominance) occurs. What is required is that when the synaptic strength of one input grows, the strengths of the other one shrinks. This can be achieved by imposing the constraint that \( \sum_{i} w_{i} \) should be kept constant (synaptic normalization). At each time interval \( \Delta t \), following a phase of Hebbian learning, in which \( w_{i}(t + \Delta t) = w_{i}(t) + \Delta w_{i}(t) \), the new synaptic strengths are forced to satisfy the normalization constraint.

Another approach for achieving competition is to modify equation 6. With Equation 6, only increases in synaptic strength can take place; decreases in synaptic strength are brought about by enforcing synaptic normalization afterwards. Both increases in synaptic strength (long term potentiation, or LTP) and decreases in synaptic strength (long term depression, or LTD) can be obtained if we assume that the postsynaptic activity level \( y(t) \) must be above some threshold \( \theta_{y} \) to achieve LTP and otherwise yield LTD; for the presynaptic activity level \( x_{i}(t) \), a similar possibility can be assumed (53). Thus:

\[ \Delta w_{i}(t) \propto [y(t) - \theta_{y}][x_{i}(t) - \theta_{x}]\Delta t \]  
[Eq. 7]

A stable mechanism for ensuring that when some synaptic strengths increase, others must correspondingly decrease (i.e., competition) is to make one of the thresholds variable. If the threshold \( \theta_{x} \) increases sufficiently as the postsynaptic activity \( y(t) \) or synaptic strength \( w_{i}(t) \) (or both) increases, conservation of synaptic strength can be achieved (53). Similarly, if the threshold \( \theta_{y} \) increases faster than linearly with the average postsynaptic activity, then the synaptic strengths will adjust to keep the postsynaptic activity near a set point value (54).

Yet another mechanism that can balance synaptic strengths is based on a form of (experimentally observed) long-term synaptic plasticity that depends on the relative timing of pre- and postsynaptic actions potentials (spike timing-dependent plasticity, or STDP) (55). Presynaptic action potentials that precede postsynaptic spikes strengthen a synapse, whereas presynaptic action potentials that follow postsynaptic spikes weaken it. STDP has the effect of keeping the total synaptic input to the neuron roughly constant, independent of the presynaptic firing rates (56).

11.4.2. Competition Through Dependence on Shared Target-Derived Resources

Keeping the total synaptic strength onto a postsynaptic cell constant (synaptic normalization) is a biologically unrealistic way of modeling competition. In both the neuromuscular and the visual system, the total number of synapses onto a postsynaptic cell increases during competition, as the winning axons elaborate their branches and the losing axons retract branches. Synaptic normalization is too rigid a constraint compared with the plasticity of the developing nervous system, and models based on this constraint may, therefore, become too restricted in the range of phenomena they can produce (57,58). If Hebbian learning rules are modified only to enforce competition and not to represent a possible physiological mechanism, this is equally unsatisfactory. Modeling the actual mechanism of competition can give the models more flexibility.
and potentially a larger explanatory and predictive power. It will also be easier to interpret and extend these models, because its variables and parameters are more directly linked to biological processes and mechanisms.

If the dependence of axons on the same target-derived neurotrophic factor is modeled, competition between input connections does not have to be enforced, but comes about naturally. In most existing models of competition for target-derived neurotrophic factor, there is a fixed amount of neurotrophin that becomes partitioned among the individual synapses or axons; i.e., there is no production, decay, and consumption of the neurotrophin (48). This assumption is biologically not realistic. The model by Van Ooyen and Willshaw (59) considers the production and consumption of neurotrophin and incorporates the dynamics of neurotrophic signaling (such as release of neurotrophin, binding kinetics of neurotrophin to receptor, and degradation processes) and the effects of neurotrophins on axonal growth and branching. The model can also incorporate the effects of electrical activity: postsynaptic activity can influence the release of neurotrophin, while presynaptic activity can influence the number of neurotrophin receptors (see the section below describing the model). The approach by Van Ooyen and Willshaw has similarities to that by Elliott and Shadbolt (60) and Jeanprêtre et al. (61), although Elliott and Shadbolt (60) does not model all the processes involved in a dynamic fashion, and Jeanprêtre et al. (61) has to assume a priori thresholds, as well as to postulate a positive feedback rather than derive it from the underlying biological mechanisms (see further below).

The model by Van Ooyen and Willshaw (59). Important variables in the model are the total number of neurotrophin receptors that each axon has and the concentration of neurotrophin in the extracellular space. In the model, there is a positive feedback loop between the axon’s number of receptors and amount of bound neurotrophin. Unlike in the work by Jeanprêtre et al. (61), this positive feedback, which enables one or more axons to outcompete the others, was derived directly from underlying biological mechanisms. Following binding to receptor, neurotrophins can increase the terminal arborization of an axon (52,62–70) and, therefore, the axon’s number of synapses. Because neurotrophin receptors are located on synapses, increasing the number of synapses means increasing the axon’s total number of receptors. Thus, the more receptors an axon has, the more neurotrophin it will bind, which further increases its number of receptors, so that it can bind even more neurotrophin, at the expense of the other axons.

Instead of increasing the terminal arborization of an axon, neurotrophins might increase the axon’s total number of receptors by increasing the size of synapses (71) or by up-regulating the density of receptors (72).

Description of the model. The simplest situation in which we can study axonal competition is a single target at which there are $n$ innervating axons, each from a different neuron. Each axon has a number of terminals, on which the neurotrophin receptors are located (see Fig. 8A). Neurotrophin is released by the target into the extracellular space, at rate $\sigma$, and is removed by degradation, with rate constant $\delta$. In addition, at each axon $i$, neurotrophin is bound to receptors, with association and dissociation constants $k_{a,i}$ and $k_{d,i}$, respectively. Bound neurotrophin (the neurotrophin–receptor complex) is also degraded, with rate constant $p_i$. Degradation of the neurotrophin–receptor complex also removes receptor molecules; therefore, new unoccupied receptors need to be in-
serted (at rate $\phi_i$) into the axon terminals. In addition, there is turnover of unoccupied receptors, with rate constant $\gamma_i$. Thus, the rates of change of the total number $R_i$ of unoccupied receptors on axon $i$, the total number $C_i$ of neurotrophin–receptor complexes on axon $i$, and the extracellular concentration $L$ of neurotrophin are:

$$\frac{dC_i}{dt} = (k_{a,i} LR_i - k_{d,i} C_i) - \rho_i C_i$$  \hspace{1cm} [Eq. 8]

$$\frac{dR_i}{dt} = \phi_i - \gamma_i R_i - (k_{a,i} LR_i - k_{d,i} C_i)$$  \hspace{1cm} [Eq. 9]

$$\frac{dL}{dt} = \sigma - \delta L - \sum_{i=1}^{n} (k_{a,i} LR_i - k_{d,i} C_i) \right / \nu$$  \hspace{1cm} [Eq. 10]

where $\nu$ is the volume of the extracellular space. The term $(k_{a,i} LR_i - k_{d,i} C_i)$ represents the net amount of neurotrophin that is being bound to receptor. Equations 8 and 9 are similar to the ones used in experimental studies for analyzing the cellular binding, internalization, and degradation of polypeptide ligands such as neurotrophins (73).

The biological effects of neurotrophins (all of which, as explained above, can lead to an axon getting a higher total number of receptors) are triggered by a signaling cascade that is activated upon binding of neurotrophin to receptor (17). In order for the total number of receptors to increase in response to neurotrophin, the rate $\phi_i$ of insertion of receptors must be an increasing function, $f_i$ (called growth function), of $C_i$. To take into account that axonal growth is relatively slow, $\phi_i$ lags behind $f_i(C_i)$ with a lag given by:

$$\tau \frac{d\phi_i}{dt} = f_i(C_i) - \phi_i$$  \hspace{1cm} [Eq. 11]

where the time constant $\tau$ for growth is of the order of days. Setting immediately $\phi_i = f_i(C_i)$ does not change the main results. Different classes of growth functions were studied, all derived from the general growth function:

$$f_i(C_i) = \frac{\alpha_i C_i^m}{K_i^m + C_i^m}$$  \hspace{1cm} [Eq. 12]

Depending on the values of $m$ and $K_i$, the growth function is a linear function (Class I: $m = 1$ and $K_i \gg C_i$), a Michaelis-Menten function (Class II: $m = 1$ and $K_i \gg C_i$), or a Hill function (Class III: $m = 2$). Within each class, the specific values of the parameters $\alpha_i$ and $K_i$, as well as those of the other parameters, will typically differ between the innervating axons, e.g., as a result of differences in activity or other differences. For example, increased presynaptic electrical activity can increase the axon’s total number of receptors (e.g., by up-regulation [74,75] or by stimulating axonal branching [26]) which implies that, for example, $\alpha_i$ is increased or $\gamma_i$ is decreased.

The whole model thus consists of three differential equations for each axon $i$ (Equations 8, 9, and 11) and one equation for the neurotrophin concentration (Equation 10). By means of numerical simulations and mathematical analysis, we can examine the outcome of the competitive process. Axons that at the end of the competitive process have no neurotrophin ($C_i = 0$, equivalent to $\phi_i = 0$) are assumed to have withdrawn or
**Fig. 8.** The model by Van Ooyen and Willshaw (59) (see Subheading 11.4.). (A) Target cell with three innervating axons, each with a different degree of branching. The target releases neurotrophin, which binds to neurotrophin receptors at the axon terminals.

For three different classes of growth functions, (B–D) show the development of innervation for a system of five innervating axons, where each axon has a different competitive strength, $\beta_i$ (defined in Subheading 11.4.). The values of $C_i$ are in number of molecules. Panels (E–G) show
died, while axons that do have neurotrophin \((C_1 > 0, \text{ equivalent to } \phi_1 > 0)\) are regarded as having survived.

All parameters in the model have a clear biological interpretation. For the numerical simulations, the parameter values were taken from the data available for nerve growth factor (NGF) (see also [59]). Mathematical analysis [59] shows that the results do not depend on specific choices for the parameters and are, therefore, also relevant for other neurotrophic factors.

**Results of the model.** For class I, starting with any number of axons, elimination of axons takes place until a single axon remains (single innervation) (Fig. 8B,E). The axon that survives is the one with the highest value of the quantity \(\beta = \frac{[k\alpha(\alpha/K_1 - \rho)]}{[\gamma_d(k + \rho)]}\) which is interpreted as the axon’s competitive strength. For class I, the number of surviving axons cannot be increased by increasing the rate \(\sigma\) of release of neurotrophin: the higher amount of neurotrophin results in further growth of the winning axon and thus more uptake of neurotrophin, so again not enough neurotrophin is left to sustain the other axons. This shows that the widely held belief that competition is a consequence of resources being produced in limited amounts is too simplistic. If the growth function is a saturating function (classes II and III), then more axons may survive if the rate \(\sigma\) of release of neurotrophin is increased (Fig. 8C,D,F,G). A saturating growth function means that the “size” of an axon (in terms of number of neurotrophin receptors) is bounded, so that when an axon is at its maximum, a higher amount of neurotrophin does not result in further growth and more uptake, so that other axons can profit.

For classes I and II, there is, for a given choice of the parameter values, only one stable innervation pattern (either single or multiple innervation). For class III, in contrast, stable equilibria of single and multiple innervation can coexist, and which of these will be reached in any specific situation depends on the initial conditions (Fig. 8D,G).

**Fig. 8. (continued)** The nullcline pictures for a system of two innervating axons [the variables \(R_i, C_i, i = 1, 2\) and \(L\) are set at quasisteady state; in (E) and (F), \(B_1 > B_2\); in (G), \(B_1 = B_2\). In (E–G), the bold lines are the nullclines of \(\phi_1\), and the light lines are the nullclines of \(\phi_2\) (the x- and y-axes are also nullclines of \(\phi_2\) and \(\phi_1\), respectively). Intersection points of these lines are the equilibrium points of the system. A filled square indicates a stable equilibrium point; an open square indicates an unstable equilibrium point. Vectors indicate direction of change. (B) Class I. Elimination of axons takes place until a single axon remains. The axon with the highest value of the competitive strength, \(\phi_1\), survives. (C) Class II. For the parameter settings used, several axons survive. (D) Class III. Dependence on initial conditions; although axon one has the highest value of the competitive strength, axon two survives because its initial value of \(\phi_1\) is sufficiently higher than that of axon one. (E) Class I. The nullclines do not intersect at a point where both axons coexist. (F) Class II. The nullclines intersect at a point where both axons coexist. For a sufficiently lower rate of release of neurotrophin, for example, the nullclines would not intersect, and only one axon would survive. (G) Class III. There is a stable equilibrium point where both axons coexist, as well as stable equilibrium points where either axon is present [the stable equilibrium point at \((\phi_1 = 0, \phi_2 = 0)\) is not indicated, because it is too close to another unstable point]. For a sufficiently higher value of \(\mathcal{K}_i\), for example, the stable equilibrium point where both axons coexist would disappear. From (48) with permission.
For all classes, axons with a high competitive strength $\beta_i$ survive, and the activity dependence of $\beta_i$ (e.g., via $\alpha_i$) means that these are the most active ones, provided that the variation due to other factors does not predominate.

The coexistence of several stable equilibria for class III implies that an axon that is removed from a multiply innervated target may not necessarily survive ("regenerate") when replaced with a low number of neurotrophin receptors (Fig. 9A,B). To enhance the possibility that a damaged axon can return and survive on its former target, the model suggest that it is more efficient to increase the number of receptors on the regenerating axons than to increase the amount of neurotrophin (which also makes the already existing axons on the target "stronger").

Comparison with empirical data. The model can account for the development of both single and multiple innervation following a stage of hyperinnervation. Examples of single innervation are the innervation of skeletal muscle fibers (9), autonomic ganglion cells with few dendrites (76), and the climbing fiber innervation of cerebellar Purkinje cells (77). Although undergoing a reduction in innervation, most other cell types remain multiply innervated. In agreement with the model, increasing the amount of target-derived neurotrophin delays the development of single innervation (class I) (78) or increases the number of surviving axons (classes II and III) (e.g., in epidermis [79]).

The model can also explain the coexistence of stable states of single and multiple innervation (class III) in skeletal muscle. Persistent multiple innervation is found in denervation experiments after reinnervation and recovery from prolonged nerve conduction block (80). In terms of the model, conduction block changes the sizes of the basins of attraction of the equilibria (via changes in the competitive strength $\beta_i$ or in the rate $\sigma$ of release of neurotrophin), so that the system can go to an equilibrium of multiple innervation, while under normal conditions single innervation develops. Once the conduction block is removed, the system will remain in the basin of attraction of the multiple innervation equilibrium (Fig. 9C,D).

For competition to occur, it is not necessary that there is presynaptic or postsynaptic activity or that there is activity-dependent release of neurotrophin (cf. [51]). Differences in competitive strength ($\beta_i$) between axons can arise also as a result of differences in other factors than presynaptic activity, such as intrinsic differences in neurotrophic signaling (e.g., insertion or degradation of neurotrophin receptors). Thus, both presynaptic and postsynaptic activity may be influential but are not decisive (81,82). This is in agreement with recent findings in the neuromuscular system, which show that activity is not necessary for competitive synapse elimination (82); silent synapses can displace other silent synapses.

The model can be, and is (Ribchester, R.R., personal communication), tested experimentally. The model predicts that the axons that are being eliminated will have a small number of neurotrophin receptors. The shape of the growth function, which determines what innervation can develop, can be determined experimentally in vitro by measuring, for different concentrations of neurotrophin in the medium, the total number of terminals of an axon or, better, the axon’s total number of neurotrophin receptors that it has over all its terminals. In relating axon survival to neurotrophin concentration, the
Fig. 9. The implications of the coexistence of stable states of single and multiple innervation for class III in the model by Van Ooyen and Willshaw (59) (see subheading 11.4.). In (A and B), removal of an axon from a multiply innervated target and subsequent replacement, for (A) class II and (B) class III. At $t = 504h$, axon 1 (bold line) is removed by setting $\alpha_1 = 0$. At $t = 756h$, axon 1 is replaced by setting $\alpha_1$ back to its original value, with initial conditions $\phi_1 = 30$, $R_1 = \phi_1 / \gamma$, and $C_1 = 0$. Only for class II the replaced axon can survive. For class III, in order for the replaced axon to survive, a much higher initial value of $\phi_1$ would be required. From (59) with permission. The phase-space plots of (C and D) illustrate how, for class III, persistent multiple innervation can arise after recovery from nerve conduction block, in a system of two innervating axons. For explanation of nullclines and symbols, see Figure 8 (for clarity, the unstable equilibria are not indicated). The triangles mark the starting points of trajectories (bold lines). As shown in (C), under normal conditions, with electrically active axons that have a different level of activity (represented by $\alpha_1 = 400$ and $\alpha_2 = 300$; other parameter values as in Fig. 8G) and a low initial number of receptors (i.e., $\phi_i$ is low: $\phi_1 = \phi_2 = 0.25$), single innervation develop. When activity is blocked (values of $\alpha_i$ lower and the same: e.g., $\alpha_1 = 250$ and $\alpha_2 = 250$), as in (D), the same initial conditions lead to multiple innervation. Subsequent restoration of activity means that the nullclines are again as in (C), but now the starting values of $\phi_i$ are those reached as in (D), i.e., in the basin of attraction of the polyneuronal equilibrium point. The system goes to this equilibrium and will remain there forever, i.e., persistent polyneuronal innervation. Another way in which persistent multiple innervation can arise following nerve conduction block is through altering the rate of release of neurotrophin, $\sigma$, which also changes the sizes of the basins of attraction of the equilibria. From (48) with permission.
model predicts, for example, that the smaller the value of $K_f$ of the growth function, the lower the concentration of neurotrophin needed to rescue more axons.

**Further extensions of the model.** In the model as described above, it is assumed that the concentration of neurotrophin is uniform across the extracellular space, so that all axons “sense” the same concentration. This is a good assumption if all the axons are close together on the target structure, as, for example, at the endplate on muscle fibers (83). However, if the target structure is large (e.g., a large dendritic tree), the spatial dimension of the extracellular space should be taken into account. Modeling local release of neurotrophin along the target and diffusion of neurotrophin in the extracellular space, Van Ooyen and Willshaw (84) showed that distance between axons mitigates competition, so that if the axons are sufficiently far apart on the target, they can coexist (i.e., even under conditions, e.g., a class I growth function, where they cannot coexist with a uniform extracellular space). This can explain that (i) when coexisting axons are found on mature muscle cells, they are physically separated (85–87), and (ii) a positive correlation exists between the size of the dendritic tree and the number of innervating axons surviving into adulthood (46,76,88). In the ciliary ganglion of adult rabbits, for example, neurons that lack dendrites are innervated by a single axon, whereas neurons with many dendrites are innervated by the largest number of axons. In newborn animals, in contrast, all neurons are innervated by approximately the same number of axons.

In another extension of the model, Van Ooyen and Willshaw (84) considered a single target that releases two types of neurotrophin (89–91) and at which there are two types of innervating axons. Each axon type can respond to both neurotrophin types, but with different affinities (e.g., each axon type may have a different type of neurotrophin receptor, with each receptor type binding to both types of neurotrophin, but with a different affinity). The results show that different types of axons can coexist (i.e., even under conditions, e.g., a class I growth function, where they cannot coexist with a single type of neurotrophin) if they respond to the neurotrophins with sufficiently different affinities. By having axons respond with different affinities to more than one type of neurotrophin, the model can account for competitive exclusion among axons of one type while at the same time there is coexistence with axons of another type innervating the same target. This occurs, for example, on Purkinje cells (77), where climbing fibers compete with each other during development until only a single one remains, which coexists with parallel fibers innervating the same Purkinje cell.

### 11.5. DISCUSSION

Stochastic dendritic growth models appear to be successful in describing the shapes of dendritic branching patterns, as shown in section 11.2, and by other authors (92–94). The parameter values, obtained after a process of optimization, are assumed to reflect basic characteristics of the branching process. Emphasis has been given to competitive phenomena as becoming apparent by the size-dependent branching probabilities. We have shown that the “competition parameter” $E$ significantly differentiates between different cell types. Competitive interactions were also suggested by Nowakowski et al. (92) as underlying a suppression of further branching immediately after a branching event.
Other successful approaches for reconstructing dendritic complexity are based on stochastic algorithms, in which segment lengths and diameters are obtained by sampling the observed distributions of shape characteristics directly (95,95). These approaches do not include a phase of parameter optimization.

For a further interpretation of the results of stochastic models in terms of underlying mechanisms, one needs to model elongation and branching at more detailed levels (such as in Subheading 11.3.). In Subheading 11.3.2., we have introduced a model that explicitly includes some of the cellular mechanisms involved in elongation and branching. In the model, we have studied the consequences of the interactions between the calcium dynamics in dendritic trees and the effects of calcium on MAP (de)phosphorylation (which influences elongation and branching). With respect to producing the variability in dendritic morphologies, the model compares well with the stochastic model (Subheading 11.2.) Reproducing the data (particularly the terminal length data) using the stochastic model required separate phases of elongation–branching and elongation only, with different rates of elongation in each phase. These phases emerge automatically in the MAP model, in which both elongation and branching are generated from the same intrinsic mechanism and need not be manipulated independently.

In Subheading 11.3.1., we have shown that competition between growing neurites can emerge as a result of the interactions between the transport of tubulin and the tubulin-mediated elongation of neurites. The model can account for “dormant growth cones” and for recent experimental findings in tissue culture (G.J.A. Ramakers, unpublished results) that show that when one neurite stops growing out, other neurites, after a delay, start growing out. These results are also relevant for understanding the formation of nerve connections, because it shows that changes in the growth of a subset of a neuron’s neurites (e.g., as a result of changes in electrical activity, or as a result of neurites finding their targets) can affect the growth of the neuron’s other neurites (see also [97]).

At their target, axons from different neurons compete for target-derived resources. Our model of axonal competition suggests that the regulation of axonal growth by neurotrophins is crucial to the competitive process in the development, maintenance, and regeneration of nerve connections. Among the many axonal features that change during growth in response to neurotrophin (degree of arborization and, consequently, number of axon terminals; size of terminals; and density of receptors), the consequent change in the axon’s total number of neurotrophin receptors, thus changing its capacity for removing neurotrophin, is what drives the competition. The form of the dose–response curve between neurotrophin and axonal arborization (or better, the total amount of neurotrophin receptors) determines what patterns of innervation can develop and what the capacity for axon regeneration will be.

REFERENCES

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