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Theoretical analysis of a synaptotropic dendrite growth mechanism

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Abstract

It is generally believed that the genome cannot encode explicit instructions to form each synaptic connection in the nervous system, but may provide general neurite growth mechanisms which will result in proper connectivity. Recent in vivo imaging has provided evidence for a synaptotropic growth mechanism, wherein synapses could influence dendrite growth by selectively stabilizing filopodia upon which they form. We undertook a theoretical investigation into the consequences of such a growth process. Discrete stochastic simulations demonstrate that the synaptotropic mechanism can result in decreased dendritic wiring length, is capable of searching for regions of high density pre-synaptic partners, and can recapitulate specific patterns of dendrite growth and connectivity. A mean-field analysis shows that growth by selective stabilization of filopodia can be approximated as a reaction–diffusion system, with a spatially varying diffusion constant that depends on the probability of synapse formation. Thus, growth will occur faster in regions of appropriate synaptic connections, and the net growth can be shown to climb a gradient of synaptic partner density. Synaptotropic growth thus presents a mechanism for the emergent development of connectivity based on local properties of the circuit elements, rather than explicit dependence on global guidance molecules or innate predetermined branching programs.

Keywords: Dendrite growth; Synapse formation; Mean-field analysis; Filopodia

1. Introduction

In order to develop proper synaptic connectivity, a neuron extends a dendrite which allows it to contact pre-synaptic partners. The molecular and cellular mechanisms determining dendritic extension and branching are beginning to be elucidated, from cytoskeletal processes underlying growth, to the intrinsic and extrinsic signals which drive dendritic arborization (Cline, 2001; McAllister, 2000; Scott and Luo, 2001; Whitford et al., 2002). However, at a higher level there is the question of how these basic mechanisms integrate into a growth process that results in a dendrite with the proper extent and connectivity. In this paper we attempt to understand this integration by exploring the consequences of one particular growth rule, the synaptotropic mechanism.

The "synaptotropic hypothesis" was first proposed based on fixed tissue studies of rat spinal cord (Vaughn, 1989). Two particular observations, the presence of synapses on growth cone filopodia (Vaughn et al., 1974), and the fact that dendrites did not extend into a region until pre-synaptic partners had arrived (Vaughn et al., 1988), supported the idea that formation of a synaptic contact could stabilize particular filopodia, biasing growth in that direction, or preventing growth in the absence of contacts. Recent in vivo imaging has provided further support for such a mechanism, by allowing a direct view of dendrite growth and synapse formation in the zebrafish tectum (Niell et al., 2004). Fig. 1a summarizes the growth process that was observed. A growing dendrite extends and retracts many small, motile processes known as filopodia, many of which persist for only a few to tens of minutes. These filopodia are similar in appearance to those found on the tips of axonal growth cones, although they can extend from mid-branch, and are occasionally stabilized leading to net growth of the arbor. Furthermore, new synaptic contacts with nearby axons are predominantly formed on

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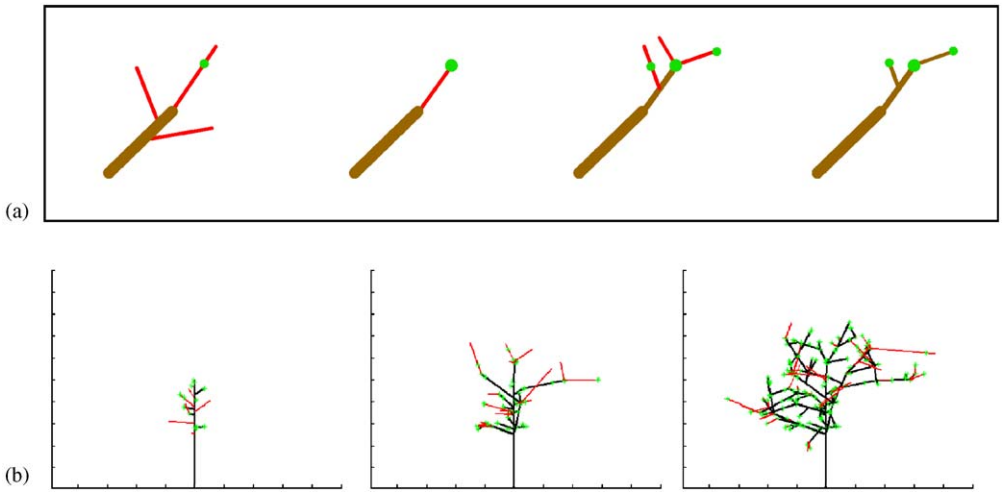

Fig. 1. Synaptotropic dendrite growth. (a) Schematic of synaptotropic dendrite growth, based on in vivo imaging of zebrafish optic tectum. Many filopodia are extended (red), but only those which form synapses (green) are maintained as stable branches. (b) Growth sequence for stochastic simulation of synaptotropic dendrite growth for spatially homogeneous synapse formation. Dynamic filopodia in red, stable branches in black, and synapses in green.

these dendritic filopodia. The small subset of filopodia which do form synapses are stabilized and persist as new branches, from which further growth occurs.

Intuitively, this growth mechanism provides a means to match the extent of a dendrite to the set of synaptic connections it forms, i.e. there are no branches without synapses. However, the formation of new filopodia by extending from previously stabilized filopodia also suggests a positive feedback, which could play a more direct role in guiding dendrite growth. To make this model explicit, we abstracted the synaptotropic growth process to three basic assumptions, and explored the dynamics of growth and patterns of dendritic arborization that result.

Previous models of dendrite growth (reviewed in Kiddie et al., 2005; van Pelt et al., 2003) have used probabilistic statistics of branching to predict graph properties of arbors, as well as the effects of synaptic activity on these statistics, and then compared the results to measurements of mature arbors. In fact, early studies of the synaptotropic mechanism used such models to predict arc lengths and the number of dichotomous and trichotomous branches (Berry et al., 1975), which were verified for Purkinje neurons, both in wild-type mice and *weaver* and *staggerer* mutants which have reductions in the number of pre-synaptic axons (Bradley and Berry, 1976). Other models have studied subcellular processes, such as cytoskeletal dynamics (Van Veen and Van Pelt, 1994) and intracellular signaling (Graham and van Ooyen, 2004; Hentschel and Fine, 1996) to mechanistically predict these local branching properties. At a higher level of organization, there have been studies of how chemical gradients and synaptic activity can result in map formation (reviewed in Willshaw and Price, 2003), although generally without taking into account the actual dendritic structures underlying the map. Our study attempts to bridge these two levels, to show how a specific dendritic growth mechanism can result in particular spatial patterns of connectivity. Furthermore, our models explore the impact of dynamic filopodial extension and retraction revealed by live imaging (Wong and Wong, 2000), rather than treating dendrite elaboration as a monotonic outward growth. We also explore the efficiency of these mechanisms in terms of arbor length and search for synaptic partners.

Discrete stochastic simulations enabled us to study the geometric and topological aspects of arbor development, while a mean-field analysis led to the mathematical demonstration of several key features of synaptotropic growth, including the equivalence to reaction–diffusion and the ability to grow up a gradient. By including additional processes, such as different forms of negative feedback to limit growth, we have the potential to increase the realism of the model, as well as to identify key parameters and constraints which are necessary to completely describe dendrite development. Overall, we hope to demonstrate the ability to start with simple local growth rules and deduce emergent global properties of the dendritic arbor and synaptic connectivity.

2. Methods

2.1. Discrete simulation

A discrete stochastic simulation was implemented in Matlab (Mathworks). We restricted analysis to two-spatial dimensions for simplicity. The simulation consisted of three types of objects—stable branches, dynamic filopodia, and synapses. New filopodia were formed on stable branches with probability p_{filo} per branch length per time. Filopodia extended at a rate v^+, and retracted at a rate v^-. They could transition once from extension to retraction with a probability p_{tr} per time. During extension, a synapse could form on a filopodium with probability p_{syn} per length

extended. During retraction, if a filopodium retracted to the point of a synapse, the filopodium was converted to a stable branch. If it retracted to zero length, the filopodium was eliminated.

The initial condition for a simulation was one stable branch extending into a region of non-zero synapse formation probability. This makes explicit the fact that we are not attempting to simulate long-range pathfinding, but arborization into or within a target region. One other geometrical parameter was the angle at which new filopodia extend from a branch. This angle was determined randomly, but was restricted to less than $\pi/2$, simply so that simulated arbors appeared more natural, although this had no effect on the global properties of growth. For the experiments described here, simulations were terminated when a given number of synapses had been formed, although other criteria would provide a test of various feedback mechanisms which may serve to limit growth (see Section 4).

To provide a basis for comparison, we also implemented two non-synaptotropic mechanisms, based on the same general structure. In the simple non-synaptotropic model, we relaxed the requirement that filopodia bear a synapse, so that once a filopodium made the transition to retraction, it was immediately converted to a stable branch. In the non-synaptotropic model with pruning, the growth process continued as per the simple non-synaptotropic model; however, once growth was stopped, terminal branches that did not bear synapses were removed. This was repeated on newly exposed terminal branches until no more branches could be eliminated.

To describe the trajectory of simulated growth, we calculated the centroid of the newly formed arbors as the mean position of the midpoint of newly formed branches, weighted by the branch length. We also calculated a radius of the arbor based on isocontours of branch density, for comparison with predictions from theory. This was computed by discretizing space and computing the local density of branch tips. The radius of the outermost isocontour was then defined as the average distance from the arbor centroid for all points with a density of one branch tip per spatial bin.

3. Results

3.1. Discrete simulation

Our discrete simulation was based on three basic assumptions from observations of synaptotropic growth. (1) Filopodia form stochastically from stable branches. (2) Synapses form on filopodia with a probability proportional to the length they extend, since the probability of encountering an axonal partner increases with the distance explored by the filopodium. (3) The presence of a synapse stabilizes a filopodium as a new branch, from which further growth can occur. We thus have a well-defined model, where all assumptions are made explicit. We should note that events that are modeled as probabilistic processes, such as (1) and (2), are not truly ''random'', but simply viewed as stochastic due unspecified conditions (e.g. intracellular signaling cascades, locations of axons, etc.)

An example of the growth process generated by the simulation, with a spatially homogeneous probability of synapse formation p_{syn}, is shown in Fig. 1b. (See also Supplemental Movie 1). Initial values for the growth parameters were based on qualitative observation of tectal cell dendrite growth (Niell et al., 2004), i.e. filopodia persist about 10 min ($p_{tr} = 0.1\,\mathrm{min}^{-1}$), they grow to about 5 μm ($v^{+} = 0.5\,\mu\mathrm{m}/\,\mathrm{min}$), approximately one quarter of filopodia form synapses ($p_{syn} = 0.05\,\mu\mathrm{m}^{-1}$, from here on normalized to $p_{syn} = 0.25$ per average filopodial length), and there are generally only a couple of filopodia on a branch at a given time ($p_{filo} = 0.2\,\mathrm{min}^{-1}$). Because we were hoping to deduce general principles of growth, rather than recapitulate specific growth rates, the actual values of these parameters are not critical. However, our simulation does allow us to test quantitatively the effect on growth rate varying these parameters. For example, Fig. 2a shows the radius of the arbor as a function of time, for $p_{syn} = 0.25$. Because this is linear, the boundary of the arbor is extending at

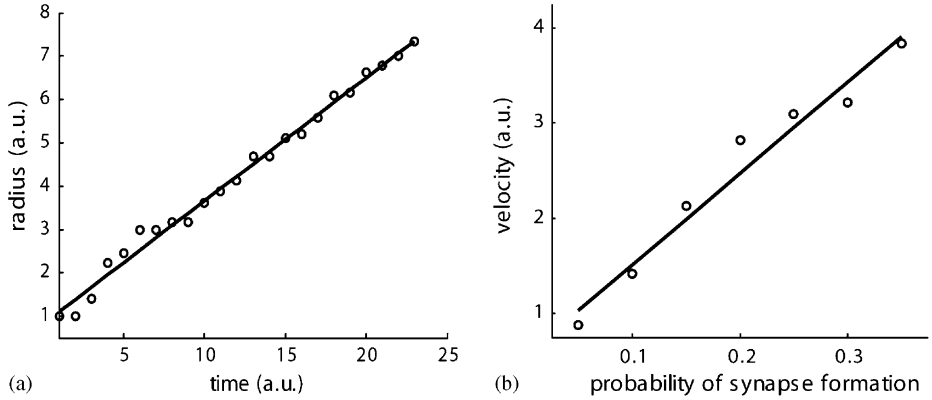

Fig. 2. Growth rates for spatially homogeneous synapse formation. (a) Radius of dendritic arbor, as determined by isocontours of branch density, averaged over 25 simulations, with best-line fit to demonstrate constant velocity. (b) Velocity of arbor expansion for different values of synapse formation probability p_{syn} shows linear relationship ($n = 25$).

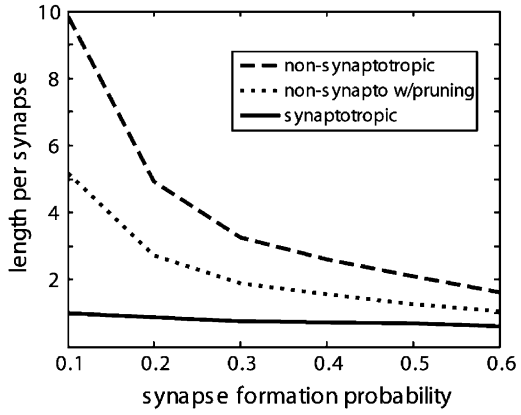

Fig. 3. Dendritic arbor length per synapse for three different growth mechanisms. Non-synaptotropic (dashed line), non-synaptotropic with pruning (dotted line), and synaptotropic (solid line). Arbor length and probability per length are normalized to the average filopodial length.

approximately constant velocity, given by the slope. Fig. 2b shows this velocity for different values of p_{syn}, demonstrating that the arbor extends more quickly when the probability of synapse formation is higher. In a later section we derive analytical expressions for the relations among such parameters.

In this model of spatially homogeneous synapse formation we can test the effectiveness of the synaptotropic mechanism in reducing dendritic wiring length. As a baseline for comparison, we used a non-synaptotropic model, where filopodia were stabilized regardless of whether they had formed a synapse, resulting in a monotonic outgrowth process. As an intermediate model, we created a non-synaptotropic model with post-pruning, wherein once growth was completed, branches without synapses were eliminated. The arbor length needed to generate a given number of synapses for these three models is shown in Fig. 3, as a function of the probability of synapse formation p_{syn}. The length of the synaptotropic arbor is approximately constant across the range of p_{syn}, because only filopodia with synapses are stabilized, and until p_{syn} becomes very high it is unlikely that more than one synapse will form on a given filopodium. At low p_{syn} the non-synaptotropic arbor is an order of magnitude larger due to the many branches which do not bear synapses. Even a post-pruning step is not able to restore the minimal size arbor, as there are many low-order branches without synapses that cannot be pruned due to higher-order branches extending from them. At high p_{syn}, though, the non-synaptotropic models become closer to the synaptotropic, because when every filopodium is likely to form a synapse, there are fewer unnecessary branches.

3.2. Generation of specific arborization patterns

One direct predicted consequence of the synaptotropic model is that it will restrict dendrite arborization to match the distribution of synaptic partners. In the simulation we can test this by having p_{syn} vary at different spatial locations. Fig. 4 shows an example where p_{syn} is zero everywhere except in two horizontal bands. (See also Supplemental Movie 2). This could represent a laminated structure where pre-synaptic axons are present in two layers. As the simulation demonstrates, even though filopodia form everywhere, only those within the two synaptic layers are stabilized. Growth then spreads out through the two layers, but does not extend beyond them. Although this is almost a trivial consequence of our basic assumptions, this anecdote provides a powerful demonstration that a defined dendritic structure does not need to result from an explicit global branching program, but could simply result from a local, stochastic process based on synapse selectivity.

3.3. Short-range guidance

There is a positive feedback inherent in the synaptotropic growth mechanism, since filopodia that form synapses can then give rise to further growth. To test whether this feedback can actually result in directed growth of the arbor, we performed a simulation with a Gaussian spatial distribution of p_{syn} (Fig. 5 and see Supplemental Movie 3). Such a situation would result if pre-synaptic partners were spatially localized, with the offset of the primary branch due either to errors in the initial pathfinding, or simply if the pathfinding does not fully specify the final target region. As shown in Fig. 5a–e, although initial filopodial extension is randomly distributed, more filopodia are likely to be stabilized closer to the target region. As this process is repeated, the arbor eventually grows towards the maximum of p_{syn}, effectively resulting in directed growth up the gradient. Fig. 5f, which plots the path of the arbor centroid for multiple trials, shows that this behavior is robust despite the stochastic nature of the growth process.

Obviously, the length of filopodia will affect the ability to find a target region. To explore the relation between target distance and filopodial length, we determined the total time needed to generate 300 synapses while varying these two factors relative to the width of the Gaussian distribution σ. Because filopodial length is an emergent property in our model, we manipulated the extension and retraction probability to vary the average length. Fig. 6a shows that there is a relatively sharp cutoff for target distance beyond which the time required to grow increases dramatically. In fact beyond this distance the arbor often does not climb up the gradient, but simply forms synapses with low probability around the initial branch. However, Fig. 6a shows that longer filopodia do indeed increase the range of search, and in general can result in guidance to a target much further than the length of an individual filopodium.

To determine the significance of this guidance, we compared the synaptotropic model with the non-synaptotropic mechanism, which will simply grow isotropically

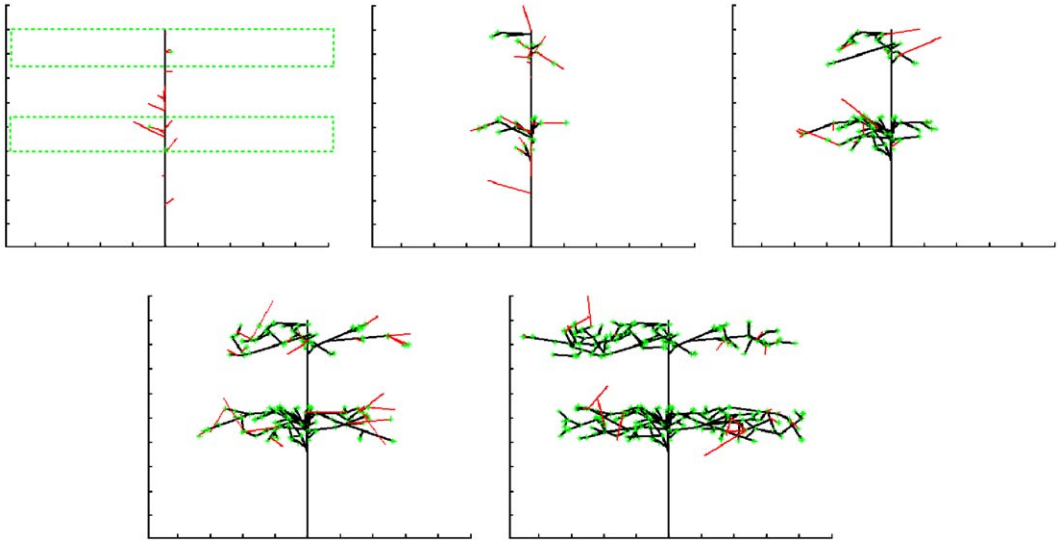

Fig. 4. Growth sequence for synaptotropic dendrite growth with non-homogeneous synapse formation. The probability of synapse formation is non-zero only in two horizontal layers (dashed boxes), and the resulting arbor growth matches this distribution.

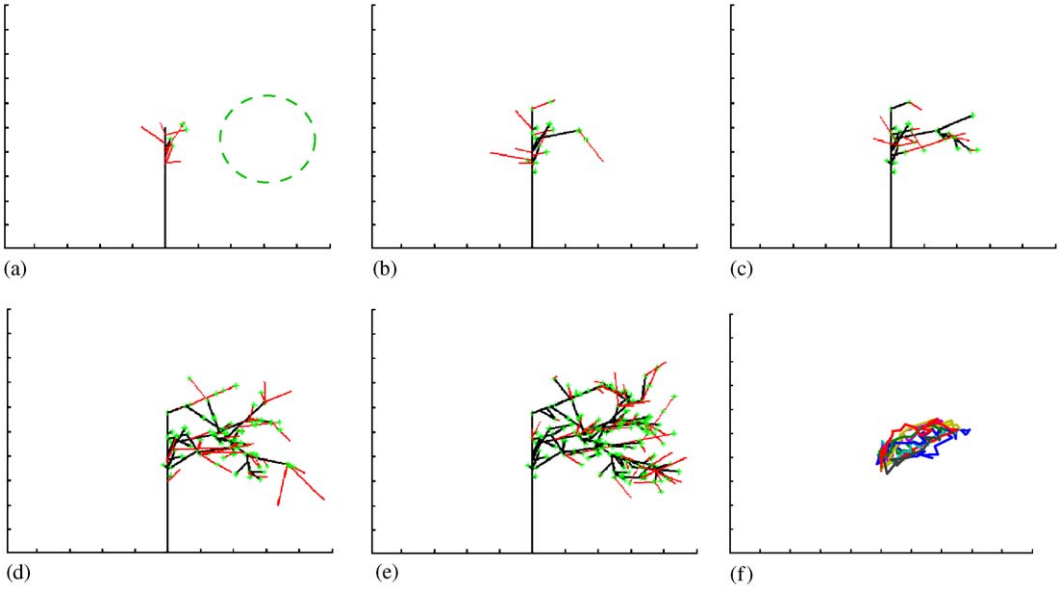

Fig. 5. (a–e) Time sequence for synaptotropic growth towards a local maximum. p_{syn} follows a Gaussian distribution, with the 1σ contour shown by green dashed line. (f) Path of centroid of new branch formation, for 10 different simulations.

because synapses do not influence their growth. We separated the effects of guidance from wiring length discussed above by calculating the ratio of the total number of filopodia extended (whether stabilized or not) for both growth processes. This measure of relative efficiency was determined for a target region at different distances from the initial branch (Fig. 6b). Even when the initial branch directly overlaps the target region (zero distance), the synaptotropic mechanism is more effective (i.e. relative efficiency >1) because it keeps the arbor constrained to regions of high p_{syn}. As the target region moves further away, the guidance provided by the synaptotropic mechanism greatly increases the efficiency in generating connectivity, so that distant targets require more than 5 times as many filopodial extensions to form the same number of synapses. Beyond a certain distance, however, even the synaptotropic mechanism does not completely climb the gradient to the maxium of the target region, and no further increase in efficiency occurs.

3.4. Derivation of growth parameters

In order to analytically assess the impact of fundamental growth parameters on arbor development, we calculated several derived quantities from the fundamental parameters that went into the simulation. If filopodia perform a

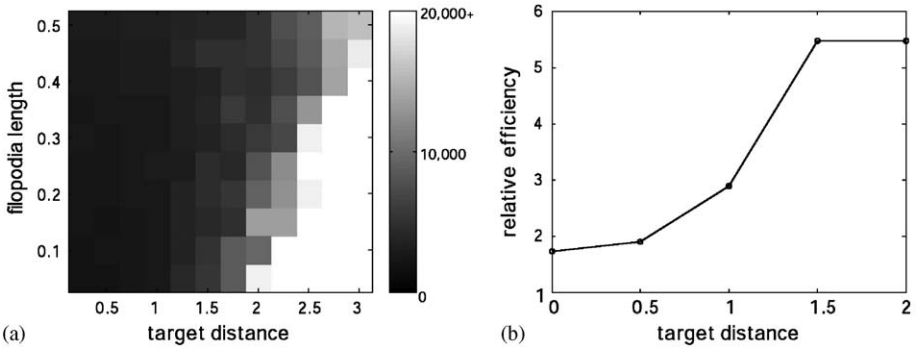

Fig. 6. Characterization of growth toward a target region. (a) Time required to form 300 synapses, for a given distance to target and average filopodial length. Lengths and distances are normalized to the width σ of the target region. Average of $n = 25$ for each point. (b) Relative search efficiency for synaptotropic versus non-synaptotropic growth for different target distances, measured as the ratio of total filopodial extensions required to form 300 synapses, $n = 25$.

single transition extension–retraction process, which is a standard model for cytoskeletal dynamics, then given extension rate v^+ and transition to retraction probability p_{tr}, the lengths of maximum extension will be distributed exponentially with a mean

$$\langle L_f \rangle = \frac{v^+}{p_{tr}}. \tag{1}$$

An approximately exponential distribution of filopodial lengths, although with an exclusion of very short filopodia, has been verified experimentally in several systems (Jontes et al., 2000; Portera-Cailliau et al., 2003).

If synapses are formed on filopodia with a probability p_{syn} per length extended, then the number of synapses formed on a filopodium with length L_f will follow a Poisson distribution with mean

$$\langle n_{syn} \rangle = p_{syn} L_f. \tag{2}$$

Given the distribution of filopodial lengths and synapse formation probabilities, one can calculate the probability of stabilization for a filopodium

$$p_{st} = \frac{\langle n_{syn} \rangle}{1 + \langle n_{syn} \rangle} = \frac{p_{syn} \langle L_f \rangle}{1 + p_{syn} \langle L_f \rangle}. \tag{3}$$

This equation gives some insight into the dependence of wiring efficiency on p_{syn} shown in Fig. 2. At low synapse formation probabilities, very few filopodia are stabilized in the synaptotropic mechanism because most do not form synapses, meaning the non-synaptotropic is generating many branches without filopodia. Even when $\langle n_{syn} \rangle$ is greater than one, though, there is still a significant fraction that do not form synapses and therefore are not stabilized.

3.5. Mean-field approximation

In order to understand the spatial dynamics of growth, we make a mean-field approximation. Rather than considering branches as discrete units, we describe the arbor in terms of a mean density of stable branches $n(\vec{x}, t)$ and filopodia $f(\vec{x}, t)$. We can then write a continuous differential equation to describe the generation of stable branches as the density of filopodia times their probability of stabilization p_{st} which was derived above as

$$\frac{\mathrm{d}n}{\mathrm{d}t} = f(x,t) p_{st}(x). \tag{4}$$

Although for conciseness and clarity of exposition the equations in this section are all written in one dimension, their extension to three dimensions is straightforward.

Because we assume that filopodia form isotropically from branches with probability p_{filo}, we can write the density of filopodial formation as a convolution integral about the branches

$$f(x,t) = \int n(y,t) w(x-y)\,\mathrm{d}y. \tag{5}$$

If the distribution of filopodial lengths is exponential, as above, then the convolution kernel $w(d)$ is simply a decreasing exponential normalized to p_{filo},

$$w(d) = p_{filo} \mathrm{e}^{-|d|/L_f}. \tag{6}$$

However, the only assumptions about $w(d)$ that are needed for this derivation are that its spatial moments

$$w_n = \frac{1}{n!} \int y^n w(y)\,\mathrm{d}y \tag{7}$$

are decreasing with n, and that it is symmetric about the origin, which implies that $w_n = 0$ for odd n.

Combining Eqs. (4) and (5), and taking advantage of the fact that $w(d)$ is symmetric to commute the convolution, gives the differential equation for branch density

$$\frac{\mathrm{d}n}{\mathrm{d}t} = p_{st}(x) \int n(x-y,t) w(y)\,\mathrm{d}y. \tag{8}$$

Following the derivation presented in Murray (2002), we express n as a Taylor expansion to give

$$\frac{\mathrm{d}n}{\mathrm{d}t} = p_{st}(x) \int w(y) \left[n(x) + y \frac{\partial n}{\partial y} + \frac{y^2}{2} \frac{\partial^2 n}{\partial y^2} + \cdots \right] \mathrm{d}y. \tag{9}$$

This can be rewritten in terms of the non-zero spatial moments of $w(d)$ from Eq. (7) to give

$$\frac{dn}{dt} = p_{st}(x)\left[w_0 n + w_2 \frac{\partial^2 n}{\partial x} + \cdots\right]. \quad (10)$$

Keeping the first two terms and evaluating the moments w_0 and w_2 for Eq. (6) gives

$$\frac{dn}{dt} = r(x)n + D(x)\frac{\partial^2 n}{\partial x^2}, \quad (11)$$

where

$$r(x) = w_0 p_{st}(x) = \langle L_f\rangle p_{filo} p_{st}(x) \text{ and}$$
$$D(x) = w_2 p_{st}(x) = \langle L_f\rangle^3 p_{filo} p_{st}(x). \quad (12)$$

Eq. (11) is in the form of a reaction–diffusion equation, where in this case both the reaction term r and the diffusion rate D vary spatially, depending on the probability of filopodial stabilization, and thus by Eq. (3) on the probability of synapse formation. This allows one to understand the patterns of arbor growth observed in simulations (Figs. 4 and 5) as diffusive extension of stable branches, where regions of high synapse formation result in a large diffusion constant and thus rapid growth.

Although there is not a simple solution to Eq. (10) with spatially varying parameters, in the case of spatial homogeneity and a localized initial arborization, it can be solved analytically as (Okubo and Levin, 2001)

$$n(x,t) \propto \frac{1}{\sqrt{Dt}} \exp\left\{rt - \frac{x^2}{4Dt}\right\}. \quad (13)$$

Contours of constant n propagate outwards at a velocity given asymptotically by

$$v \propto \sqrt{rD} = p_{filo} p_{st} \langle L_f\rangle^2 \quad (14)$$

allowing us to predict the spatial extension of the arbor in terms of filopodial and synapse formation parameters. From Eq. (3), for low $\langle N_{syn}\rangle$ we can make the approximation $p_{st} \approx p_{syn}\langle L_f\rangle$, giving

$$v \propto p_{filo} p_{syn} \langle L_f\rangle^3. \quad (15)$$

This agrees with the dependence of growth velocity on synapse formation determined from stochastic simulations (Fig. 2a,b), and provides a mathematical justification for the prediction that the arbor will extend more quickly through regions of high synapse formation. Note also the supra-linear dependence on average filopodial length, due to the fact that (a) longer filopodia are more likely to form synapses and be stabilized, (b) once stabilized they give rise to a greater number of subsequent filopodia, and (c) they cover a larger distance thus increasing the rate of arbor extension, each of which essentially contributes a linear term.

One can also demonstrate that the diffusion term will contribute to growth up a gradient toward the region of maximum synapse formation probability, by calculating the velocity of the centroid which, assuming for simplicity that n is normalized to integrate to unity, is given by

$$\langle \vec{v}\rangle = \frac{d\langle \vec{x}\rangle}{dt} = \frac{d}{dt}\int \vec{x} n(\vec{x})\, dV. \quad (16)$$

Moving the derivative inside the integral and applying the diffusion term from Eq. (11) in vector form gives

$$\langle \vec{v}\rangle = \int \vec{x} D(\vec{x}) \nabla^2 n(\vec{x})\, dV. \quad (17)$$

This can be integrated by parts and thereby, assuming $n = 0$ at the spatial boundaries, gives to first order in $D(x)$

$$\langle \vec{v}\rangle = \int n(\vec{x}) \nabla D(\vec{x})\, dV. \quad (18)$$

In other words, the velocity of the centroid is equal to the weighted average of the diffusion coefficient gradient, thereby directing growth towards the regions of maximum synapse formation probability. Thus, the guidance exhibited in Fig. 5 can be seen as a general property of the synaptotropic mechanism, directly resulting from the reaction–diffusion framework. Similar guidance could result if the other parameters in D, such as p_{filo} or L_f, vary spatially. Several studies have demonstrated signaling mechanisms, including synaptic activity, that affect filopodial formation (Li et al., 2000; Maletic-Savatic et al., 1999) so our model would predict guidance for these systems as well.

4. Discussion

4.1. Properties of synaptotropic growth

One general approach to modeling is the *in silico* reconstitution experiment, attempting to reproduce a specific set of experimental observations using appropriate parameters. Although our models would permit such an analysis given a measured set of parameters, we chose to pursue the opposite conceptual direction, instead attempting to abstract and determine general capacities of the synaptotropic growth process. We began with three "postulates" about the basic growth mechanism, based on in vivo imaging . From these, discrete simulations demonstrated the geometric and topological properties of branch patterns. On the other hand, a mean field analysis left out many physical arbor properties, but allowed us to give a mathematical explanation of the net spatial dynamics of the arbor, and an intuitive understanding of directed growth as a reaction–diffusion system.

Several recent studies have investigated the relationship between synaptic connectivity and wiring optimization (Chklovskii, 2004; Chklovskii et al., 2002). We therefore examined the efficiency of the synaptotropic growth mechanism in terms of reducing branch length. While this is a straightforward result of the basic assumptions, we show that it is significantly more effective even compared with a mechanism where branches are pruned once growth is complete. We also demonstrate quantitatively the regime

of synapse formation in which the effect is significant, illustrating how different growth rules may be appropriate depending on the particular synaptogenic problem to be solved.

Fig. 4 demonstrates the ability of the synaptotropic mechanism to generate specific arborization patterns based solely on the distribution of pre-synaptic partners. Of course, to some extent this just pushes back the basic patterning issues to the localization of axons. However, it does demonstrate that once a spatial pattern is produced, subsequent growth processes can be based upon it, rather than each element relying on an intrinsic program to generate morphology. Furthermore, if such a synaptotropic mechanism operates on both sides, it could facilitate self-organization which would refine these initial patterns.

We also show that the process of local branch stabilization can result in the emergent property of guidance over longer length scales, effectively seeking out inhomogeneity in the distribution of pre-synaptic partners. To go beyond anecdotal demonstration as in Fig. 5, we developed a mathematical framework by showing that synaptotropic growth can be formalized as a reaction–diffusion equation, to show that this guidance is an inherent property of this mechanism. Furthermore, the explicit mathematical description allows an examination of factors which affect differentially affect the rate of extension and the guidance behavior.

The ability to analyse dendrite growth as a reaction–diffusion system could be quite useful, as this framework has been extensively studied in a wide range of applications, particularly in population biology, as well as for various forms of pattern formation, from chemical reactions to animal coat patterns (Murray, 2002). Many of the extensions one might make to our dendrite growth model correspond to known problems which have been solved in other fields. For example, implementing the physical limitation of a maximum number of synapses per volume is isomorphic to a maximum carrying capacity for animal populations, which is implemented by including a logistic term $1-n/N_{max}$ in Eq. (11). Likewise, limiting the duration over which branches can extend filopodia is analogous to a limited reproductive cycle for animal populations, which can be implemented by adding an age dimension to n. The dependence of growth on synaptic activity can be implemented by adding an extra differential equation for local synaptic activity s, with cross-terms between branch formation and synaptic activity, which would yield systems of differential equations similar to those for interacting populations of multiple species. All of these systems have been well-characterized in the context of population biology (Okubo and Levin, 2001).

4.2. Interaction with other growth mechanisms

Although we implemented the synaptotropic mechanism in isolation, so that we could demonstrate that it is inherently capable of generating patterned growth, it is most likely that several processes operate together to create a specific dendritic arbor. For example, a genetically encoded branching pattern (Komiyama et al., 2003; Moore et al., 2002) may lay out the basic arbor, with synaptotropic growth filling in higher-order branches. In fact, the arbor growth could be almost completely defined by other mechanisms, with the synaptotropic growth simply serving as a "proof-reading" to eliminate erroneous branches, or to provide robustness as the positive feedback will tend to push it back towards high-density regions. Furthermore, rather than considering a single neuron in isolation, a model that incorporates many neurons with the possibility of lateral interactions could give insight into how local growth processes affect the formation of large-scale topographic maps.

Likewise, it is apparent that many dendrites do not use a synaptotropic mechanism. For example, isolated hippocampal neurons can form elaborate dendrites in vitro without pre-synaptic partners (Banker and Cowan, 1979), and molecular mechanisms have been found which contribute significantly to arbor elaboration that act cell-autonomously (Moore et al., 2002). (Also for general reviews of dendrite growth see Jan and Jan, 2003; Scott and Luo, 2001). Our analysis hopefully sheds some light on those situations in which a synaptotropic mechanism would be productive, versus those in which even if it exists, it would not have an effect on arbor growth, such as when the density of synaptic partners is high or when they are homogeneously distributed. In fact, in some cases synaptotropic growth may be a liability—the inherent positive feedback will tend to push the arbor towards high-density regions, even if this is not the target region specified by other growth processes. Future studies incorporating multiple growth mechanisms should allow the relative contributions of different processes to the final arbor to be teased apart.

4.3. Experimental verification

The utility of a model lies not only in its ability to explain previous results, but in the ability to generate specific predictions. One obstacle to testing this model is that a crucial set of boundary conditions, the specificity and spatial distribution of pre-synaptic partners, is not known for most systems. In the absence of this distribution, one can generate a number of "just so" stories to describe the formation of different arborization patterns based on hypothesized pre-synaptic distributions. However, as the layout of different neural circuits is elucidated, given the pre-synaptic distribution and measurement of a few growth parameters, such as filopodial formation rate and average length, one could use our model to predict the eventual dendritic arbor, which could be compared to the actual arbor which results. As different cell types are studied, it should be possible to determine the relative contribution of the synaptotropic mechanism to defining their particular morphology. One would expect that different dendritic morphologies, from the tightly localized glomerular dendrites of olfactory mitral

cells, to the broad space-filling arbors of motoneurons, may result from matching to different distributions of presynaptic partners.

Our model also has the ability to predict the results of experimental perturbations. Again, the most direct test would be perturbations that specifically affect synapse formation. In particular, locally removing a set of synaptic partners could result in a diffusion barrier, which would inhibit growth, and expression of ectopic partners would effectively attract the arbor. Simpler gross manipulations, such as reducing synapse density or filopodial lengths, would also have quantitative results predicted by our model which are not always intuitive. For example, reducing p_{syn} by a factor of two would decrease the rate of extension commensurately, as given by Eqs. (3) and (14), but would generate an arbor with approximately the same number of synapses per length, as shown in Fig. 2.

Making precise predictions of an arbor (in silico reconstitution) will be difficult because the synaptotropic mechanism is likely not the only, or even dominant, mechanism operating in the growth of a particular dendrite. However, as more growth determinants are elucidated, our simple model can be made more realistic. Both frameworks we have presented are particularly amenable to extension to multiple mechanisms. For example, an external chemical cue that directs growth can be added explicitly in the discrete model, or included as a drift term in the reaction–diffusion model. Different signaling mechanisms that influence growth, such as rho GTPases or synaptic activity, could be included as spatial and temporal variation of the basic growth parameters such as p_{filo} or p_{st}.

Furthermore, a major gap in our knowledge is the signals which limit growth or end the developmental phase, which could have dramatic effects on the final arbor form. For example, if growth duration is determined by a specific time interval, then reducing synapse formation would result in smaller arbors, due to the decreased growth rate in Eq. (15). On the other hand, if the arbor grows until it reaches a total amount of synaptic input, then although the growth rate is lower, the final arbor could look identical even if the synapse formation rate were reduced. Such processes can be incorporated into models to understand the interaction between the positive feedback of synaptotropic growth, and negative feedback which limits growth. Quantitative models such as these should allow an understanding of how individual mechanisms result in emergent growth processes, such as the guidance to target regions that could result from the synaptotropic rule studied here, which can then be combined into a mathematical framework for understanding the generation of morphology and connectivity in the nervous system.

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Appendix A. Supplementary Materials

The online version of this article contains additional supplementary data. Please visit doi:10.1016/j.jtbi.2005.11.014.

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