Accurate Reconstruction of Neuronal Morphology

by Jaeger (2001)

CPSC 644

Presented by Yoonsuck Choe

Need for Accurate Morphological Reconstruction

- Dendrite diameter of 0.8 µm, estimated to be 0.5 µm will result in 60% error in surface area and 156% for cross-sectional area.
- Thus, small errors like that can result in huge differences in physiological simulations.
- Many sources of error:
  - Ignoring dendritic spines
  - Shrinkage during histological processing
  - Optical limit

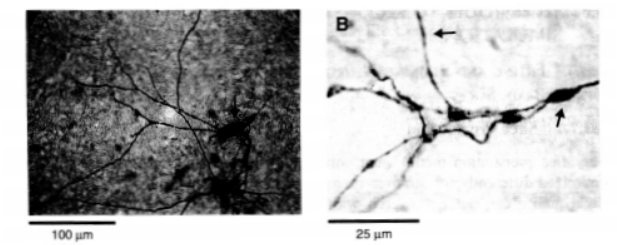
Techniques

- Stain during intracellular recording: Inject biocytin/neurobiotin followed by coupling to avidin-HRP. Dark stain results. Motorized stage/microscope used for reconstruction.
- Fluorescent dyes can also be used, but hard to reconstruct.

Filling and Staining Neurons in Slices

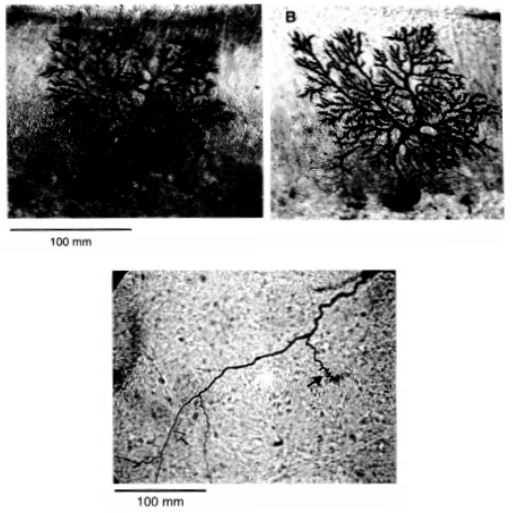
- Slice preparation
- Injection of biocytin
- Fixation of slices
- Histological processing of slices
- Mounting and clearing of thick slices

Uniformity Issues



- Quality of staining is not uniform: Some cells are fine, some are not.

Problems with Slice Preparation

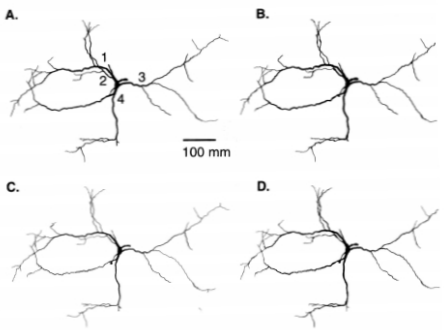


- Distortion and shrinkage.
- Curled up parts.

Other Methods for Neuronal Morphology Acquisition

- Photoconversion of fluorescent dyes (selective tagging possible)
- Golgi method: dark staining of full neurons, but only a small number of neurons are stained. However, large number of samples can be obtained, compared to injection methods.
- Filling individual neurons in fixed tissue
- Electron-microscopy: dendrites and spines can be measured with high accuracy.
- High-voltage EM tomography: 3D imaging.
- Confocal microscopy

Tracing Neurons under LM



- Resolution: $0.6 \times \lambda/\mathrm{NA}$. For λ = 500 nm and 1.0 numerical aperture (NA), resolution limit is 0.3 μm.
- Moving stage plus manual reconstruction software is used to reconstruct neurons (tracing one neuron takes about 30 minutes to several days).
- Individual variations in tracing results

Variation in Reconstruction

TABLE 6.1
Cell Statistics of Four Reconstructions of the Same GP Neuron

	Rec. A	Rec. B	Rec. C	Rec. D
Length Dendrite 1 (μm)	1160	1226.3	1378.1	1307.1
Surface Area Dendrite 1 (μm)^2	4829.09	5230.72	4963.62	4344.62
Branch Points Dendrite 1	8	8	11	15
Length Dendrite 2 (μm)	540.6	508.4	481.3	650.6
Surface Area Dendrite 2 (μm)^2	1899.23	1931.47	1057.34	2321.12
Branch Points Dendrite 2	4	3	5	6
Length Dendrite 3 (μm)	1107.5	1158.4	1133.1	1268.7
Surface Area Dendrite 3 (μm)^2	3980.27	4204.36	2641.09	4147.13
Branch Points Dendrite 3	7	7	8	9
Length Dendrite 4 (μm)	1249.6	1251.9	1242.7	1385
Surface Area Dendrite 4 (μm)^2	5352.67	5535.01	3723.33	5007.53
Branch Points Dendrite 4	11	9	11	20

Note: Pictures of the reconstructions are shown in Figure 6.4. The surface area of the cell in particular is quite variable between reconstructions. All people performing these reconstructions had previous experience in the use of Neurolucida. Specific instructions as to how to trace thin processes were not given.

- Individual differences are apparent.

Modeling Dendritic Geometry and the Development of Nerve Connections

by van Pelt et al. (2001)

CPSC 644, Spring 2010

Presented by Yoonsuck Choe

Overview

- Model of dendritic geometry: stochastic generation by elongation and branching
- Model for the development of interneuronal connectivity: competition for neurotrophic factors.

Modeling Dendritic Geometry

- Morphology, development of morphology, and relation to neuronal connectivity are of interest.
- What are the “fundamental rules” or minimal parsimonious descriptions of architecture, development, and function?
- Reconstruction model
- Growth model

Reconstruction Model

- Measure parameters from observed data.
- Random sampling on the estimated distribution to generate synthetic neurons having the same distribution.
- Several different approaches exist (see the text).

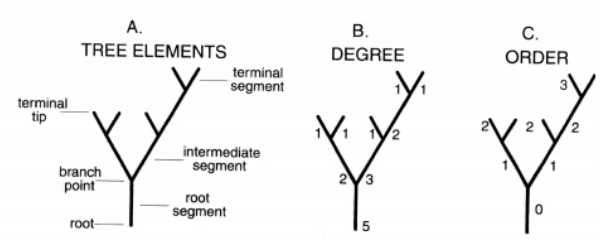
Growth Model

- Aim is to reveal rules of neuronal growth in relation to the geometric properties of the trees emerging from these rules.
- Dynamic behavior of growth cones are considered.
- Elongation and branching.
- Topological vs. metric growth models.
- Growth over time is modeled, so time-dependent aspect can be investigated.

Ingredients of Growth Models

- Choices of segments at which branching occur
- Time pattern of branching events
- Elongation of segments

Geometry of Dendritic Trees



- Number of terminal tips (degree) or branch points
- Lengths and diameters of the segments
- Connectivity pattern of segments
- Terminal vs. intermediate segments
- Path length, Centrifugal order; Asymmetry index

$$A_t(\alpha^n) = \frac{1}{n-1}\sum_{j=1}^{n-1} A_p(r_j, s_j), \quad A_p(r,s) = |r-s|/(r+s-2)$$

Dendritic Growth Model: Assumptions

- Branching at the tip of terminal segments
- Elongation only at terminal segments
- Branching parameters can be estimated from observed terminal segment number distribution.

Dendritic Growth Model

- Branching process: variation in the number of segments and the variation in topological tree types depends on
  - Number of terminal segments (or tips)
  - Expected number of branching events
  - Dependence of branching on number of tips
- Elongation process: variation in segment lengths
  - Random elongation predefined distribution
  - Intermediate segment length distribution: can be monotomically decreasing or have a modal shape
  - Branching event not a point process in time, but proceeds during a certain period of time during which a growth cone splits and the daughter branches become stabilized
- Time
- Segment diameter: $d_p^e = d_1^e + d_2^e$ with exponent e.

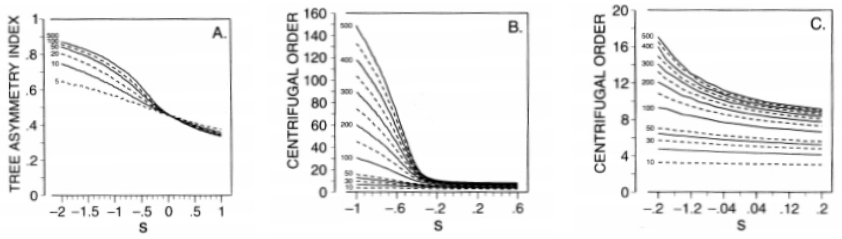
Dendritic Growth Model Parameters

TABLE 7.1
Summary of Parameters Used in the Dendritic Growth Model

Parameter	Aspect of Growth	Related to
B	Basic branching parameter	Segment number
E	Size-dependency in branching	Segment number
S	Order-dependency in branching	Topological structure
$\alpha_{l_{in}}$	Initial length—offset	Segment length
$\overline{l_{in}}$ (μm)	Initial length—mean	Segment length
$\sigma_{l_{in}}$	Initial length—SD	Segment length
$\alpha_{v_{be}}$	Elongation in "branching/elongation phase"—offset	Segment length
$\overline{v_{be}}$ (μm/h)	Elongation in "branching/elongation phase"—mean rate	Segment length
α_{v_e}	Elongation in "elongation phase"—offset	Segment length
$\overline{v_e}$ (μm/h)	Elongation in "elongation phase"—mean rate	Segment length
cv_e	Coefficient of variation in elongation rates	Segment length
$\overline{d_t}$ (μm)	Terminal segment diameter—mean	Segment diameter
σ_{d_t}	Terminal segment diameter—SD	Segment diameter
$\overline{e}$	Branch power—mean	Segment diameter
σ_e	Branch power—SD	Segment diameter

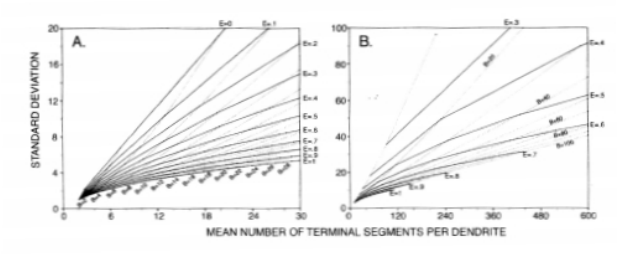
Note: Note that the segment diameter parameters are not part of the growth model, but used afterwards to assign diameter values to the skeleton tree produced by the model.

Effects of Growth Parameter S



- Each plot shows multiple plots for trees with different order.
- S: can be estimated from the value of the topological asymmetry index, or from the mean centrifugal order of the tree.

Effects of Branching Parameters B, E



Basic branching parameter B and Size-dependency of branching E can be estimated from:

- Mean number of terminal segments per dendrite
- Standard deviation of terminal segments per dendrite

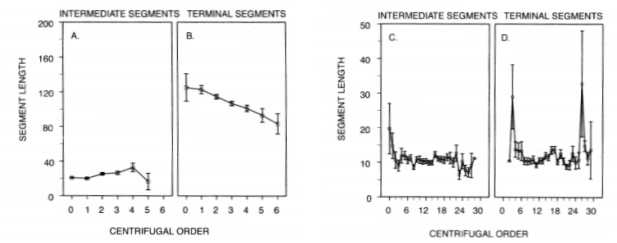
Estimation of Metric Parameters

Segment length offset α, Mean segment length $\bar{l}$, Mean elongation rate $\bar{v}$, and standard deviation of segment length σ, at three different stages:

- Initial
- Branching/elongation period
- Elongation period

Estimated obtained through optimization process.

Estimation of Elongation Rate



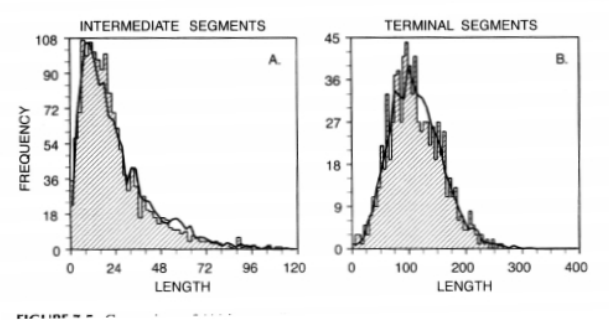
- Terminal segments are longer than intermediate segments
- Decrease in terminal segment length with increasing centrifugal order: This is affected by sustained elongation of segments and their initial lengths, thus ratio between length of lowest and highest segment can help estimate sustained elongation rate.

Other Parameters

- Variation in sustained elongation rates: Estimated by the variation in path lengths distribution.
- Diameter parameters: direct calculation

Example Results: S1-Rat Cortical Layer 2/3 Pyramidal Cell

Intermetidate and Terminal Segment Length Distribution



- Model matches the data pretty well.

Observed vs. Model

TABLE 7.2
Comparison of Shape Properties from Experimental Observations of S1-Rat Cortical Layer 2/3 Pyramidal Cell Basal Dendrites and of Model Simulated Trees

	Observed		Model Predicted	
Shape Parameter	Mean	Standard Deviation	Mean	Standard Deviation
Degree	4.04	2.04	4.05	2.02
Asymmetry index	0.41	0.24	0.4	0.23
Centrifugal order	1.86	1.2	1.85	1.19
Total dendritic length			527.6	265
Terminal length	110.7	45.2	112.62	44.8
Intermediate length	22.0	17.9	23.6	18.0
Path length	163.8	48.1	164.6	45.0

Obtained with optimized values of the growth parameters.

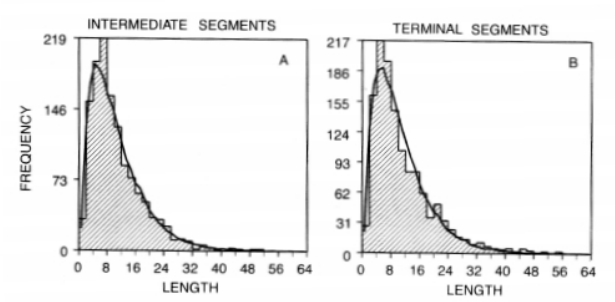
TABLE 7.3
Optimized Values for Growth Parameters to Match the Statistical Shape Properties of S1-Rat Cortical Layer 2/3 Pyramidal Cell Basal Dendrites

Growth Parameters										
B	E	S	$\alpha_{v_{tr}}$	$\overline{l_{tr}}$ (μm)	$\sigma_{l_{tr}}$	$\alpha_{v_{tr_{br}}}$	$\overline{v_{tr}}$ (μm/h)	$\alpha_{v_{tr}}$	$\overline{v_t}$ (μm/h)	cv
2.52	0.73	0.5	0	6	5	0	0.2	0	0.86	0.4

Note: Note that v_{tr} and v_t define the sustained elongation rates during the first period of branching a elongation with a duration of 312 h (13 days), and the second period of elongation only with a duratic of 96 h (4 days), respectively.

Example Results: Guinea Pig Cerebellar Purkinje Cell

Intermetidate and Terminal Segment Length Distribution



- Model matches the data pretty well.

Observed vs. Model

TABLE 7.4
Comparison of Shape Properties from Experimental Observations of Guinea Pig Cerebellar Purkinje Cell Dendritic Trees and of Model Simulated Trees

	Observed Trees 1+2+3		Model Trees	
Shape Parameter	Mean	Standard Deviation	Mean	Standard Deviation
Degree	436	31.8	436	32
Asymmetry index	0.5	0.01	0.49	0.02
Centrifugal order	13.7	5.1	13.8	5.9
Total length	9577	1105	9265	683
Terminal length	11.3	8.8	10.6	7.5
Intermediate length	10.6	7.5	10.6	7.6
Path length	189.3	64.1	166	66

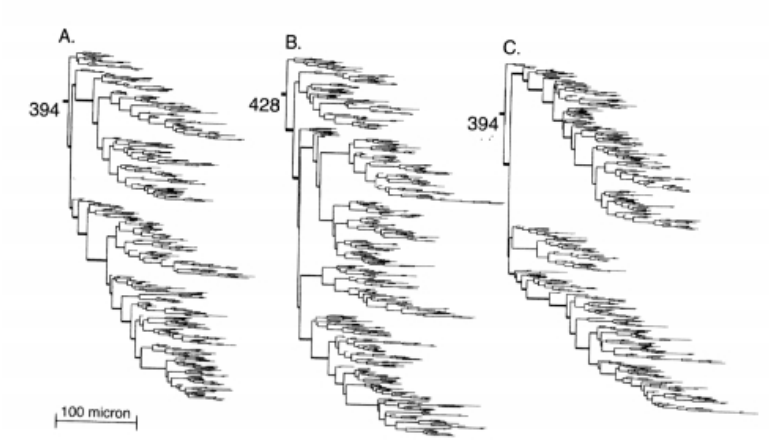
Obtained with optimized values of the growth parameters.

TABLE 7.5
Optimized Values for Growth Parameters to Match the Statistical Shape Properties of Guinea Pig Purkinje Cell Dendritic Trees

Growth Parameters					
B	E	S	α_{t_m}	$\bar{l}_m$	σ_{t_m}
95	0.69	−0.14	0.7 μm	10.63	7.53

Note: Parameters B, E, and S define the branching process, and α_{lm}, $\bar{l}_m$, and σ_{lm} define the gamma distribution for the initial segment lengths.

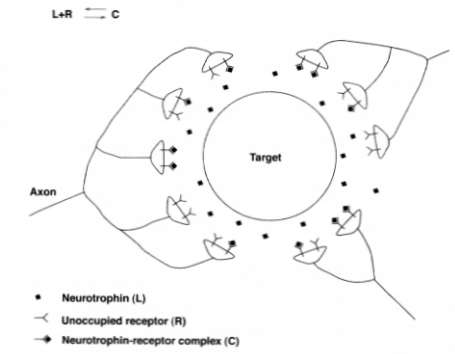
Generated Random Trees



Competition for Neurotrophic Factor in Development of Nerve Connections

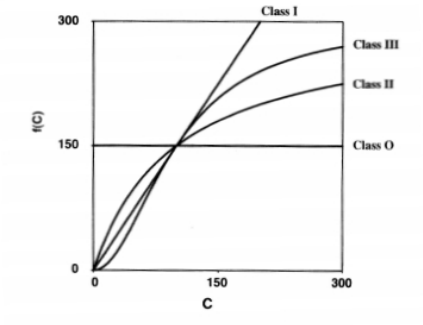
- Proliferation followed by elimination
- Single-axon or multiple-axon innervation
- Neurotrophins are involved in such growth: NGF is an example
- Competition through normalization or threshold adaptation

Neurotrophin Action at a Single Target



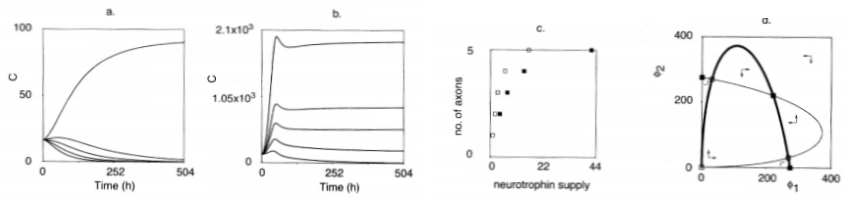
- Axonal competition at a single target
- Secretion of neurotrophin by the target
- Removal of neurotrophin: degradation, diffusion, binding (reversible)
- Number of neurotrophin receptors (NTR) C, Unoccupied NTR R, NT concentration L

Axonal Growth



- Binding triggers arborization of axons and increase in the number of axon terminals.
- Other effects include: increased size of axon terminals, upregulating NTR density, etc.
- Number of unoccupied NTR inserted ϕ
- Growth function $f(C)$ depends on number of bound NTR C.

Results and Predictions



- Single innervation: resulting number of axons
- Multiple innervation: resulting number of axons
- Rate of neurotrophin release vs. number of axons
- Coexistence of single and multiple innervation

References

Jaeger, D. (2001). Accurate reconstruction of neuronal morphology. In (Schutter 2001), chapter 6.

Schutter, E. D., editor (2001). *Computational Neuroscience: Realistic Modeling for Experimentalists*. Boca Raton, Florida: CRC Press.

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