Anat Embryol (2001) 204:255–265

REVIEW ARTICLE

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The need for integrating neuronal morphology databases and computational environments in exploring neuronal structure and function

Accepted: 9 May 2001

Abstract Neurons connect to each other through a myriad of dendritic and axonal arborisations. Dendritic structures provide the substrate for integration of postsynaptic potentials and control of action potential generation. Axonal structures provide the substrate for action potential dissemination and signalling to target neurons. The morphological complexity of dendritic arborisations is assumed to play a critical role in the transformation of spatio-temporal patterns of postsynaptic potentials into time-structured series of action potentials. Although these transformations lie at the basis of information processing in the brain, it is still far from understood how their details are influenced by dendritic shape. To facilitate research in this area, it is necessary that data on both the morphology and electrical properties of neurons, as well as computational tools for analysis, become available in an integrated way. This requires a combined effort from the fields of informatics and neurosciences (together called neuroinformatics) in order to create data acquisition, databasing and computational tools. Focusing on neuronal morphology, this chapter will give a brief review of the current neuroinformatics developments in both reconstruction techniques, morphological quantification, modeling of morphological complexity, modeling of function and the need for databasing neuronal morphologies. Additionally, one of the dendritic modeling approaches is described in more detail in the Appendix.

Keywords Neuronal morphology · Development · Neuronal function · Database · Computational neuroanatomy

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Introduction – neuronal morphology

Neurons are the principle functional elements in the electrical and chemical communication in the nervous system. With their branched processes neurons extend their surface and allow connections to be formed with thousands of other neurons each. They so form strongly connected networks, providing the substrate for brain function and information processing. Dendritic arborisations receive and integrate incoming synaptic potentials with complex spatio-temporal patterning, and trigger the neuron to generate and transmit time-structured action potentials via their axonal arborisations to local and remote target neurons. Neurons attain their shapes as the result of a developmental process in which intracellular mechanisms and interactions with local environments are operating in concert. Activity-dependent mechanisms make morphological development also a function of the neuron's connectivity and activation within the neuronal network (see for a review, e.g., van Ooyen 1994). These processes contribute to the large variations in neuron shapes between and within different cell types. Morphological alterations also occur during ageing and in neurodegenerative diseases such as Alzheimer dementia (e.g., De Brabander et al. 1998; Uylings et al. 2000).

Although it is realized that the morphological complexity of dendritic and axonal arborisations plays a crucial role in the signal transformation details of neurons and networks, and thus in information processing in the brain, it is still far from understood. A great barrier for understanding how structural details in dendritic and axonal arborisations depend on and influence neuronal function is the complexity of the system, appearing in the neuron's morphology, the electrodynamics of the membrane (dendritic trees also contain a wealth of active ion-channels and receptors, which most likely permit high degrees of local signal processing) and the spatio-temporal patterns of synaptic innervations.

What is needed are tools for accurate and efficient reconstruction of neuronal morphologies, mathematical modeling and computational tools for describing mor-

phological complexities, computational tools for studying the electrical signal transformation process on these morphologies, theories to guide the researcher in exploring the essential (and 'meaningful') aspects in spatio-temporal synaptic input patterns and time-structured spike trains, and tools for managing and communicating large amounts of data. It is the aim of the field of neuroinformatics, which combines neuroscience and informatics research, to develop and apply the advanced tools and approaches needed for studying brain structure and function at these higher levels of complexity. Examples of computational tools can be found in recent volumes in Computational Neuroscience such as Segev et al. 1995; Koch and Segev 1998; Poznanski 1999; De Schutter and Cannon 2001. They include: (1) neuron simulators, which allow, by simulation, the study of electrical signal processing and action potential generation in neurons as a function of the neuron's geometry and the innervation patterns and neuron states; (2) neural network simulators, which are indispensible for studying the bioelectric activity of groups of connected neurons and for identifying how the functional repertoire of network dynamics add to that of single neurons; (3) computational tools for studying neuronal morphological complexity.

Information technology not only contributes to tools for, so to speak, experimentation *in computo* (De Schutter 1994). An important area of application is the development of tools for the acquisition, processing, reconstruction, analysis and visualization of neuronal morphological data. Advances in imaging and visualization technologies, in particular, are expected to boost research in neuronal morphology. For instance, confocal microscopy is offering high resolution 3D digitised images revealing neuronal structures up to great detail. The technology for processing the neuronal objects in such images still needs to be developed.

Knowledge about genetic and physiological properties of individual neurons is rapidly expanding and results in a large demand for more quantitative morphological data of neurons, including data on changes in neuronal structures during development, ageing and degeneration. For the nervous system this will open exciting possibilities to link physiological function and genetic composition to morphological shape. In addition, such data will also be needed to support the fast-increasing efforts to quantitatively model the electrophysiological behaviour of individual or groups of nerve cells.

Exchange of data and integration of experimental and computational approaches are key issues in scaling up the complexity of scientific research questions and in stimulating international collaborations. Easy and integrated access to databases and research tools are in this respect indispensible. The field of neuroinformatics is able to provide the tools and approaches to meet these demands, in data acquisition, data processing, computational modeling, data storage and access, and data communication. These neuroinformatics developments will allow neuronal arborisations to be reconstructed with greater morphological detail and the signal transformation properties to be thoroughly analysed. These studies will help in extracting those degrees of freedom in input patterns, dendritic states and dendritic structure that are critically determining the time structure of the resultant output spike trains, and have 'meaning' for the nervous system context.

The next sections will focus on how neuroinformatics is involved in recent developments in neuronal reconstructions, morphological analysis, morphological modeling, electrophysiological modeling and neuronal databases.

Morphological reconstruction

Neuronal structures can be visualised by staining with a contrast substance (e.g., by means of Golgi techniques, HRP, or immunocytochemistry). Neurons can be 3-dimensionally reconstructed by manually tracing the branched structure in the microscope field and registrating the 3D coordinates of points of interests, such as cell body contour points, exit points of processes, branch points, terminal tips and points of significant curvature. Additionally, segment diameters can be measured at different locations. This method leads to skeleton reconstructions of piecewise straight segments, eventually provided with 'flesh', approximated by cylinders when diameters are measured. This procedure has been used already for decades by means of home-made systems (e.g., Glaser and van der Loos 1965; Overdijk et al. 1978; Glaser et al. 1983; Stockley et al. 1993) or commercially available systems such as has been produced by Eutectic (Capowski and Sedivec 1981; Capowski 1989, no longer in production) and by Neurolucida from MicroBrightField (http://www.microbrightfield.com/) (Glaser and Glaser 1990).

Manual tracing methods are time consuming and result in an approximated morphological reconstruction of connected cylinders. By this approximation, many structural details of the object, like neuritic irregularities and the position and shape of spiny protrusions, are mostly ignored. Reconstruction of stained neurons further puts an upper limit to the thickness of the slices. When neurons are not fully contained in a single slice, subsequent slices have to be measured followed by a serial reconstruction procedure.

Recent developments

Since the introduction of confocal microscope systems, high resolution images can be obtained of neurons stained with a fluorescent dye. Such fluorescent neurons can be reconstructed in the convential way by first treating the tissue immunocytochemically to make the neurons darkly stained and visible in the light microscope field. NeuronTracer (from Bitplane AG, http://www.bitplane.ch) has made a first attempt to reconstruct neurons directly from the digitized confocal image stacks. Still, the basic segmentation approach used needs to be fol-

lowed by an elaborate phase of image editing by an experienced operator. New developments in image analysis and visualization, however, are needed to provide crucial technologies for more sophisticated and adaptive segmentation procedures and automation of the reconstruction process of 3D neuronal arborizations from stacks of digitized (confocal) images. User interaction and control is expected to remain essential in the reconstruction process, but new technologies may reduce the user interaction down to an essential minimum, for instance by developing optimized user interfaces. When successful, this approach allows the morphological reconstruction and quantification of a neuron up to any detail of its image. Additional requirements concern storage and export facilities and compatibility with general analysis and visualization facilities (such as neural simulators). These challenges have already triggered specific research in the field of neuroinformatics. For instance, progress has been made in automatic tracing of neuritic objects in voxel fields, with centerline detection, diameter measurements and curvature measurements (Streekstra et al. 1999, 2000). Denoising techniques are being developed to filter out blur and noise without disturbing the smallest neuronal structures, using 3D wavelet transformations (Dima et al. 1999).

A different approach in imaging and reconstruction of neurons and their mutual connections is based on 3D reconstruction from stacks of images, in real time obtained from a block of tissue, from which 1-µm-thick sections are consecutively being milled off (McGormick 1999), followed by a reconstruction process from the stack of images (Barton and McGormick 1999). Advantages of such an approach are that reconstruction includes both neurons, their environments and mutual connections. Additionally, when neurons are completely contained within the scanned block of tissue, no further serial reconstructions are needed.

Morphological characteristics and variation

Neurons are three-dimensional objects and the location of their cell bodies within the nervous tissue, as well as the number, spatial extent, branching complexity and 3D embedding of their axonal and dendritic arborisations, are prominent shape characteristics that differ significantly between cell types.

Branching complexity of dendritic arborisations

The branching complexity of neuronal arborisations is characterized by topological and metrical properties. For topological characterization a neuronal arborization is reduced to a skeleton structure of points (branching or terminal points) and segments between these points. Such a skeleton forms a typical *rooted tree* out of a finite set of possible different *tree types* (van Pelt and Verwer 1983). The tree-asymmetry index provides a topological measure based on asymmetries in pairs of subtrees at bifurcations (van Pelt et al. 1992). A segment can be labeled by its *centrifugal order* (number of segments on its path to the root). Metrical aspects include *length* and *diameter* of the segments, *path lengths* (total length of the path from the dendritic root to a branch point or terminal tip), *radial distances* of terminal tips from the center of the cell and *branching angles*. Further description includes measures for the irregularity, spatial orientation and curvature of the branches. Defining a skeleton tree and assigning diameters to segments are not trivial operations. Branch points are abstract constructs representing areas where a parent branch splits in daughter branches. Especially when more than two daughter branches arise from a splitting area the observer must decide between a multifurcation point or a sequence of (close) bifurcation points with small intermediate segments in between. The use of centerlines in the parent and daughter branches may be helpful in such decisions. Segments are generally not smooth cylinders with constant diameters. The observer must decide whether a segment is represented by one single cylinder or by a series of cylinders with different diameters. Branches are generally curved structures and the observer must make a reasonable approximation with straight segments.

Spatial embedding of neuronal arborizations

A different class of measures is concerned with the spatial embedding in 3D space and focus on for instance the spatial extension, spatial density, spatial orientation and space filling of the structure. Initially such measures were developed for the projected 2D image. For instance, one can put an overlay of concentric circles on the projected image, and count the number of branch points within each circle (Sholl analysis, Sholl 1953); or one can put a cartesian grid onto the projected image and count the total projected length of branches within each grid element so to obtain a spatial resolved density measure (Ruiz-Marcos 1983; Uylings et al. 1986). Measures for orientation may be derived from principle component analysis (PCA) to find major and minor axes of a point cloud derived from the structure (see Uylings et al. 1986, 1989 for reviews; Blackstad et al. 1993). Recent studies use fractal dimensions (Takeda et al. 1992; Smith et al. 1996) or introduce the Hausdorff distance metric to construct a measure for the similarity of neuronal arborizations on the basis of their spatial structure (Mizrahi et al. 2000). All these methods generally ignore the internal connectivity structure of the neuronal arborizations, making the measures less suited for reconstructing or synthesizing random trees.

Modeling neuronal morphology

Different strategies are used in modeling the complexity and variety of neuronal arborizations. *Reconstruction*

models aim at finding minimal algorithms for generating trees which reproduce the statistical properties of observed neuronal shapes. *Growth models* aim at finding elementary rules of development to 'explain' the eventual variation in full grown arborizations. *Stochastic growth models* assume the growth actions to be described as outcomes of stochastic processes, while *mechanistic growth models* aim at describing the outgrowth process on the basis of intra- and extracellular mechanisms. All strategies aim at synthesizing neuronal branching patterns that conform as much as possible to the shape characteristics of observed neurons. Strategies differ in the assumptions made, in the meaning of the model parameters and in the procedures to find optimal values for them. Reconstruction models have been developed and are used by, e.g., Kliemann 1987, Hillman 1988, Burke et al. 1992, Tamori 1993, Uemura et al. 1995, Ascoli and Krichmar 2000, and Devaud et al. 2000. Stochastic growth models have been developed and are used by, e.g., Sadler and Berry 1983, Ireland et al. 1985, Horsfield et al. 1987, Nowakowski et al. 1992, van Pelt et al. 1997, van Pelt and Uylings 1999a, and van Pelt et al. 2001a. Examples of mechanistic growth models are given by van Veen and van Pelt 1994, Li and Qin 1996, Hely et al. 2000, and van Ooyen et al. 2001. While the algorithms in reconstruction models are directly or indirectly based on the empirically data, growth models use parameterized algorithms and require a phase of parameter optimization. This is a non-trivial step in the analysis, and the optimized parameters may directly quantify developmental processes, that are experimentally hard to approach. Examples are the predictions for the time-dependent elongation and branching rates of terminal segments during development of rat cortex pyramidal cell basal dendrites (van Pelt and Uylings 1999b). Other recent developments include the use of stochastic L-systems as algorithms for generating synthetic trees (DeVaul and McGormick 1996; Ascoli and Krichmar 2000).

Present progress in modeling neuronal morphology increasingly demonstrates the success in reproducing their complexity to a high degree of accuracy. One such development concerns the work of Ascoli and collaborators who aim at modeling neurons in their full 3D appearance, in combination with sophisticated visualization techniques (Ascoli 1999). The reconstruction approach they follow is described in detail in Ascoli et al. 2001. Their thorough discussion of the results make clear that full natural complexity is not easily captured, but also shows the promising route they follow towards their goal. van Pelt and collaborators followed a growth model approach with a step by step implementation in the course of time. First, they concentrated on the topological properties of dendritic trees and showed what branching rules were needed to explain topological variability. Second, elongation rules were included demonstrating that also length characteristics within dendrites could be accurately captured. Segment diameters were not included as part of the developmental process itself but were assigned afterwards, i.e., to the full grown skeleton tree, using a branch power relation between the segments at bifurcations. Some morphological aspects are not yet covered by the present model such as the 3D embedding and irregularity of branches, as well as the number and type of dendrites emerging from the cell body. These aspects will also be included in a step by step fashion, each time concentrating on the new morphological features and the minimal and essential model assumptions, additionally needed for their description. A brief account of this model is given in the Appendix of this article. Results will be shown for the model analysis of (basal) dendritic trees of rat cortical large and small layer 5 pyramidal cells, S1-rat cortical layer 2/3 pyramidal cells, guinea-pig cerebellar Purkinje cells and cat deep layer superior colliculus neurons. These growth model studies not only show that dendritic geometrical complexity can be reproduced accurately, but they also provide hypothetical views on the developmental process itself. For example, topological analyses showed that branching mainly occurred at terminal segments, with probabilities that depend on the centrifugal order of the segments, and decrease during outgrowth when the number of segments increases. Metrical analyses showed that growth can proceed in two phases, the first one including elongation and branching, the second one including elongation only, while the elongation rates differed between the two phases. Also needed was the assumption that newly formed daughter segments have already an initial length, suggesting that branching events include a phase of daughter segment development and stabilization. All these conclusions are made from growth model analyses, providing well-formulated quantitative hypotheses that can guide further experimental investigations for their validation.

Neuronal development proceeds by an interplay between genetic programs, intracellular mechanisms and local environmental factors. Activity-dependent mechanisms play an important role in morphological plasticity and contribute to the shaping of neurons and neuronal networks, in response to the electric activity evoked from within and outside the network. Even synaptic activity can locally induce dendritic morphogenesis (Maletic-Savatic et al. 1999). Technological progress in gene expression quantification (microarrays and DNA chips) will further provide essential data for studying the concerted actions of cell biological and gene processes in response to local environmental conditions and state of activation. Computational approaches and information technology will play a key role in the elucidation of gene networks and their topologies, in the quantification of complex cellular pathways and their implications for neuronal shape and plasticity. It may be anticipated that these approaches will provide new insight in the determinants of morphological differentiation and variety.

Computational approaches are also fruitful in showing how simple rules of develoment and/or operation may underlie a rich repertoire of seemingly unrelated phenomena and/or behavior. For instance, van Ooyen (1994) and van Ooyen et al. (1995) showed that a simple

rule of activity-dependent neurite outgrowth resulted in a variety of phenomena including overshoot, compensatory sprouting and size differences among cells.

Modeling neuronal function

The complexity of electrical signal processing in neurons has already for a long time been a major driving force in developing theoretical and computational approaches, and has been at the basis of the growing field of computational neuroscience (as witnessed by the series of CNS meetings and proceedings, and books, such as McKenna et al. 1992; Segev et al. 1995; Koch and Segev 1998); Poznanski 1999; and De Schutter and Cannon 2001).

A particular class of questions is concerned with how the electrical activity of a neuron, in terms of a time-structured series of action potentials, can be understood as a function of neuronal morphology, membrane properties and patterns of spatio-temporal synaptic innervation and distribution of post-synaptic potentials on its dendritic trees. Analytical treatments were originally used by approximating the dendritic tree by a set of connected passive cylinders (cables) and applying the so-called 'cable theory' (e.g., Rall 1959, 1977, 1995). Cable models have been successfully used in elucidating static structure-function relationships. For instance, Jack and Redman (1971) developed an electrical description of the motoneuron to investigate the effects of varying electrical and geometrical parameters on the time course of electrical transients at the soma. Barrett and Crill (1974) applied cable modeling and electrophysiological data to morphologically reconstructed cat motoneurons, enabling them to calculate the membrane capacitance and a lower limit for the membrane conductance and capacitance. Koch et al. (1982) applied passive cable modeling to formulate a functional interpretation of dendritic morphology in retinal ganglion cells. They showed that dendritic architecture of different types of retinal ganglion cells reflects characteristically different electrical properties, likely to be relevant for their physiological function and informatation processing role.

Cable models are, by their analytical treatment, restricted in incorporating time- and state-dependent membrane properties and complex innervation schemes. Compartmental models have become very popular as they do not have these restrictions. Neuronal structure is approximated by a series of compartments, (taken small enough to allow linearization) within which the interactions are described by easy to solve difference equations. Time- and voltage-dependent ion channel kinetics as well as complex dendritic innervation patterns can be solved by means of numerical techniques. This approach enabled, for instance, Shepherd et al. (1985) to investigate signal enhancement in distal cortical dendrites by means of interactions between active dendritic spines, and Miller et al. (1985) to study synaptic amplification by active membrane in dendritic spines, and Mel (1993) to study synaptic integration in an anatomically characterized neocortical pyramidal cell with active dendritic membrane and the effect of spatially clustered synaptic drive, De Schutter (1994) to study the implications of spatially inhomogenous distributions of ion channels in large scale cerebellar Purkinje cell simulations, and Mainen and Sejnowski (1996) to find firing patterns strongly correlating with the extent of arborisation. Recently, Häusser et al. (2000) reviewed particular examples illustrating diversity and dynamics of dendritic signalling, including the role of dendritic structure. Two neural simulators have become very popular, viz. Neuron (http://neuron.duke.edu/) and Genesis (http://www.bbb.caltech.edu:80/GENESIS/genesis.html), both of which are now stable products with well-developed user interfaces. These products are excellent tools for studying the implications of dendritic morphology for neuronal function.

Postsynaptic potentials change their time profile while traveling along the branches in dendritic trees and have delayed arrival at the soma (e.g., Agmon-Snir and Segev 1993; Schierwagen and Claus 2001). Therefore, both dendritic structure and the spatio-temporal pattern of innervating postsynaptic potentials play an important role in the transformation of dendritic-synaptic activity into the time structure of the generated action potentials. Spike timing is also influenced by the structure of axonal arborisations as was shown by Innocenti et al. (1994) and Tettoni et al. (1996) in a computational study of action potential propagation and spread in arrival times in reconstructed axonal arbors. The axons in this study were modeled as diameter-dependent delay lines. Evidence is accumulating for the critical role of spike timing in synaptic plasticity (e.g., Markram et al. 1997; Zhang et al. 1998). It is expected that the implication of these mechanisms for the synapses distributed over the dendritic tree strongly depend on dendritic morphology itself, by its effect on timing relations.

Where and how information is contained within neuronal firing patterns and network activity and how neurons and networks process information (neural coding) are presently key issues in computational neuroscience (see also De Schutter 2001). Clearly, the question of what role neuronal morphology plays in information processing requires understanding of the code itself. Formal information theory may be used by calculating the information content in spike trains in number of bits and study the transformation in neuronal input-output relations (e.g., Reinagel and Reid 2000). The question of which aspects in spike trains carry "meaning'', however, still holds and may not be answered without reference to "context''.

The neuronal structure-function question is of almost infinite dimensionality, that definitely requires a systematic and theory-based approach, as well as the availability of data of reconstructed neurons, and computational modeling tool.

Neuronal morphology databases

Understanding brain structure and function in health and disease can be considered as one of the great challenges

of this century. Progress in the understanding of how neurons and neuronal networks process information has long been hampered by the lack of data and tools. Recently, however, neural simulators have matured into versatile tools for structure-function studies, which are widely available and routinely used by the neuroscience community. Although the technologies to observe and record neuronal morphology and function have also increased substantially over the last decade, the ability to access, analyse, and integrate the massive amounts of data has remained extremely restricted. For instance, in the course of time many research groups have spent many hours in morphological reconstruction of neurons, but scientific publications generally do not include the raw data themselves but only the outcomes of diverse analysis procedures. Nevertheless, it may be expected that the raw data is still be present at local research sites, possibly in local formats. It would therefore be an enormous stimulation to the field if the reconstructed neurons would be made available to the scientific community (Koslow 2000). Additional to the morphological variability per se we need to know whether membrane properties are more or less invariant or scale with morphology. Raw morphological data can presently be obtained from only a few sites on the Internet. Rapp et al. (1994) made available three guinea pig cerebellar Purkinje cells at http://www.ls.huji.ac.il/~rapp/. Rat hippocampal neurons (in vivo and in vitro) can be obtained from the Southampton archive at http://www.neuro.soton.ac.uk/. This site is intended to facilitate the free exchange of data between groups studying neuronal morphology. NeuronDB (http://senselab.med.yale.edu/senselab/neurondb/) 'provides a dynamically searchable database of three types of neuronal properties: voltage gated conductances, neurotransmitter receptors, and neurotransmitter substances. It contains tools that provide for integration of these properties in a given type of neuron and comparison of properties across different types of neurons'. An integrated neuronal morphology analysis information system is being developed at http://www.bbb.caltech.edu/hbp/database.html in the context of the Human Brain Project. NeuroSys at http://nervana.montana.edu/NeuroSys/ 'combines a relational database with a set of powerful computational tools for the analysis of structure-function relationships in nervous systems. It provides a powerful and enabling tool for the formulation and testing of hypotheses related to neural computation, plasticity and development'.

Parallel with the development of widely accessible electronic databases of neuronal morphologies, it is essential to develop standards for data structures and representations, as well as facilities for visualization and analysis. Also needed are computational tools that enable researchers to analyse and synthesise the knowledge stored within a database into better understandings of the different aspects of neuronal morphology: its variability, its abnormalities in disease, its functional role in information processing, as well as its development. It is a major challenge for the field of neuroinformatics to work on these issues. A global effort has been initiated in 1996 by the OECD Megascience Forum Working Group on Biological Informatics (http://www.oecd.org/dsti/mega). Presently, the Global Science Forum Neuroinformatics Working Group works on implementation initiatives of tools and databases, guidelines for interoperability, and an internet based knowledge repository for neuroinformatics (portal). Related activities are being promoted within the EU Thematic Network Neuroinformatics (http://www.neuroinf.org).

Summary

The question of how neuronal structure and neuronal information processing are related is an incredibly complicated one, given the almost infinitely large number of degrees-of-freedom in spatio-temporal synaptic input patterns and spike trains, the (activity-dependent) adaptive mechanisms regulating membrane properties (ion channels, receptors, densities and kinetics), network connectivity and morphological properties, and the enormous variety in neuronal shapes. This means that we are still at the beginning in exploring how neuronal structure is involved in the processing of electrical signals (and the encoded information therein). This is the challenge for neuroinformatics – to provide the appropriate tools, theories and computational approaches to deal with these complexities and to find strategies for unraveling these intricacies.

This article has reviewed some of these developments in neuronal reconstruction, in modeling morphological complexity, in the simulation of electrical activity in neurons, and in databasing of neuronal morphologies.

Appendix

Dendritic growth model

The dendritic growth model aims at describing morphological complexity and variability of dendritic trees for a wide variety of neuron types. The model includes basic actions of elongation and branching of segments and assumes these actions to be stochastic. This assumption stems from the notion that the actual behavior of growth cones, mediating elongation and branching, is subject to so many intracellular and extracellular mechanisms that a probabilistic description is appropriate. The stochasticity assumption, thus, does not imply that the processes involved are stochastic by themselves, but only that their outcome can be described as such. The model has a modular structure, evolved in the course of time by studying the branching and elongation process step by step, with empirical validation after each step. The modular structure of the model also facilitates the optimization of the model parameters.

The dendritic growth model has recently been discussed in van Pelt and Uylings (1999a) and van Pelt

et al. (2001a). Briefly, the branching probability of a terminal segment during dendritic outgrowth is given by $p_i = C2^{-S\gamma}B/Nn_i^E$, with N denoting the total number of time bins in the full period of development and n_i denoting the actual number of terminal segments in the tree at time bin *i*. Parameter *B* denotes the expected number of branching events at an isolated segment in the full period, while parameter *E* determines how strong the branching probability of a terminal segment depends on the actual number of terminal segments in the tree. Parameter γ denotes the centrifugal order of the terminal segment and $C = n_i / \Sigma_{j=1}^{n_i} 2^{-S\gamma_j}$ is a normalization constant, with a summation over all n_i terminal segments. Parameter *S* determines how strong the branching probability of a terminal segment depends on the proximal-distal location of the segment in the tree. The number of time bins *N* can be chosen arbitrarily but such that the branching probability per time bin remains much smaller than one, making the probability of more than one branching event per time bin negligibly small. Newly formed daughter segments after a branching event are given a gamma-distributed, randomly chosen initial length with mean $\overline{l_{in}}$ and standard deviation $\sigma_{l_{in}}$, and a gamma-distributed, randomly chosen elongation rate. The developmental period may consist of a first phase of elongation and branching, and a subsequent phase of elongation only, with elongation rates $\overline{v_{be}}$, and $\overline{v_e}$ respectively, both with a coefficient of variation cv_v. A summary of the model parameters is given in Table 1.

The branching parameters can be derived from the shape of the empirical terminal segment number distribution. The topological structure of a fully grown dendrite is determined by the sequence of particular segments at which branching occurs. The segment lengths are determined both by the elongation rates of the segments and by the elapsed time between successive branching events. Segment length distributions can therefore only be studied once the branching process has been optimized. The computational loop for generating random trees is drawn is Fig. 1.

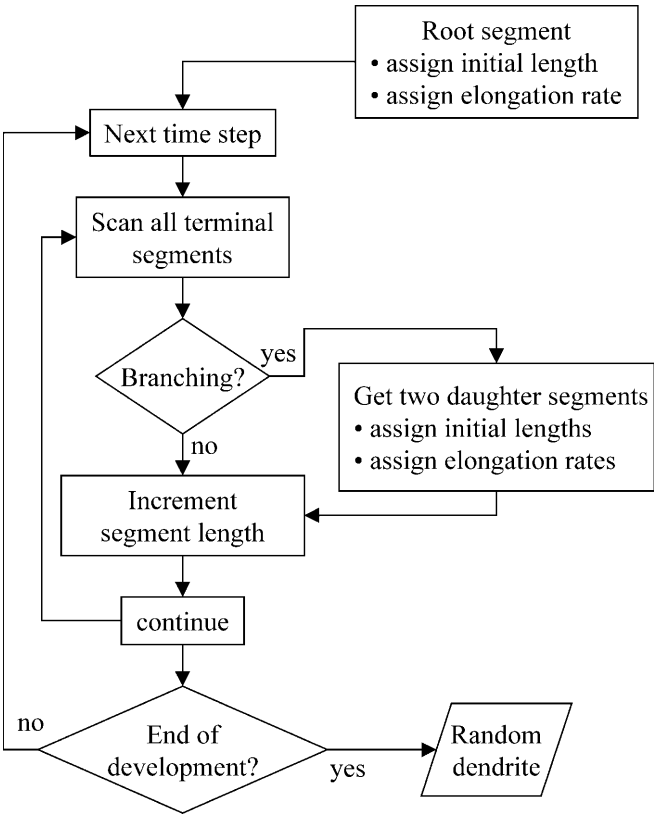

Fig. 1 Flow diagram for the generation of a random tree

Segment diameter

No developmental rules have been incorporated for the diameter of segments. Rather, these diameters are assigned to the segments of the full-grown skeleton tree. A power law relationship is assumed that relates, at a branch point, the diameter of a parent segment (d_p) to the diameter of its daughter segments d_1 and d_2 via $d_p^e = d_1^e + d_2^e$, with e denoting the branch power exponent. According to this relation, the diameter of an intermediate segment d_i relates to the number n and diameter d_t of the terminal segments in its subtree as $d_i = n^{1/e} d_t$, independent of the topological structure of the subtree. The following procedure has been used to assign diameters to the segments of the skeleton tree. First, terminal segment diameters d_t are assigned by random sampling the observed diameter distribution (or a normal distribution based on the observed mean-sd values). Then, traversing the tree centripetally, at each bifurcation the diameter of the parent segment is calculated by means of the power law relation using (a) the diameters of the daughter segments and (b) a branch power value e obtained by randomly sampling the observed branch power distribution.

Results

The model has recently been applied to dendritic trees from several neuronal types. These include basal dendrites of Wistar-rat cortical layer 5 large pyramidal neurons (van Pelt and Uylings 1999a) and of cortical layer 5 small pyramidal neurons (van Pelt and Uylings 1999b), basal dendrites of S1-rat cortical layer 2/3 pyramidal neurons (van Pelt et al. 2001a), guinea pig cerebellar Purkinje cell dendritic trees (van Pelt et al. 2001a), and of cat deep layer superior colliculus neurons (van Pelt et al. 2001b). The optimized parameter values are summarized in Table 2.

Examples of random trees are given in Fig. 2, with (A) random model trees for the "S1-rat layer 2/3 pyramidal basal dendrites'' parameter set, (B) random model trees for the "cat deep layer superior colliculus dendrites'' parameter set, and (C) a random model tree for the "guinea pig cerebellar Purkinje cells'' parameter set.

Table 1 Summary of parameters used in the dendritic growth model. A distinction is made between optimizing parameters whose values are subjected to optimization, and experimental parameters whose values are taken (in)directly from experimental observations. Note, that the segment diameter parameters are not part of the growth model, but used afterwards to assign diameter values to the skeleton trees, produced by the model. It is assumed that the gamma distributions for the elongation rates have zero offset

Optimizing parameters	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
$\alpha_{l_{in}}(\mu m)$	Initial length – offset	Segment length
$\overline{l_{in}}(\mu m)$	Initial length – mean	Segment length
$\sigma_{l_{in}}(\mu m)$	Initial length – SD	Segment length
$\overline{v_{be}}(\mu m/h)$	Mean elongation rate in 'branching/elongation phase'	Segment length
$\overline{v_e}(\mu m/h)$	Mean elongation rate in 'elongation phase'	Segment length
cv_v	Coefficient of variation in elongation rates	Segment length
Experimental parameters		
$T_0(h)$	Start of growth	
$T_{be}(h)$	End of branching/elongation phase	
$T_e(h)$	End of elongation phase	
$\overline{d_t}(\mu m)$	Terminal segment diameter – mean	Segment diameter
$\sigma_{d_t}(\mu m)$	Terminal segment diameter – SD	Segment diameter
$\overline{e}$	Branch power – mean	Segment diameter
σ_e	Branch power – SD	Segment diameter

Table 2 Parameter values of the dendritic growth model optimized for (I) basal dendrites of Wistar-rat cortical layer 5 large pyramidal neurons (van Pelt and Uylings 1999a), (II) of Wistar-rat cortical layer 5 small pyramidal neurons (van Pelt and Uylings 1999b), (III) basal dendrites of S1-rat cortical layer 2/3 pyramidal neurons (van Pelt et al. 2000a), (IV) guinea pig cerebellar Purkinje cell dendritic trees (van Pelt et al. 2001a), and (V) cat deep layer superior colliculus neurons (van Pelt et al. 2001b). Diameter parameters for cell types I and II were obtained from Larkman 1991 and Larkman et al. 1992, for cell type III from Hillman 1988 and Larkman 1991, for cell type IV from Hillman 1988, and for cell type V from Schierwagen and Grantyn 1986

Parameter	Cell type				
	I[a] Wistar rat, Large L5	II[a] Wistar rat, Small L5	III S1 rat, L2/3	IV[b] Guinea pig, Purkinje	V Cat deep layer, superior colliculus
B	3.85	3.35	2.52	95	3.89
E	0.74	0.63	0.73	0.69	0.285
S	0.87	0.87	0.5	–0.14	0.4
$\alpha_{l_{in}}(\mu m)$	–	–	0	0.7	0
$\overline{l_{in}}(\mu m)$	–	–	6	10.63	17
$\sigma_{l_{in}}(\mu m)$	–	–	5	7.53	12
$\overline{v_{be}}(\mu m/h)$	0.22	0.24	0.2		0.6[c]
$\overline{v_e}(\mu m/h)$	0.51	0.64	0.47		0.6[c]
cv_v	0.28	0.4	0.86		0.7
$T_0(h)$	–24	–24	24		0[c]
$T_{be}(h)$	240	240	336		500[c]
$T_e(h)$	432	432	432		25[c]
$\overline{d_t}(\mu m)$	0.8	0.7	0.6	1.1	1.47
$\sigma_{d_t}(\mu m)$	0.2	0.1	0.1	0.1	0.3
$\overline{e}$	1.5–2	1.5–2	1.6	2	1.05
σ_e	–	–	0.2	0.3	–

[a] The resolution of the experimental segment length distributions for cell types (I) and (II) was insufficient to optimize the initial-length parameters. The elongation rates for these cell types have thus been optimized for the situation that daughter segments had no initial length at the time of branching

[b] The initial length assignments appeared to be sufficient to describe the segment length distributions in cell type (IV). No further sustained elongation was needed for optimally matching the observed length distributions

[c] The developmental period as well as the elongation rates for cell type (V) have been expressed in time units Δt of arbitrary length

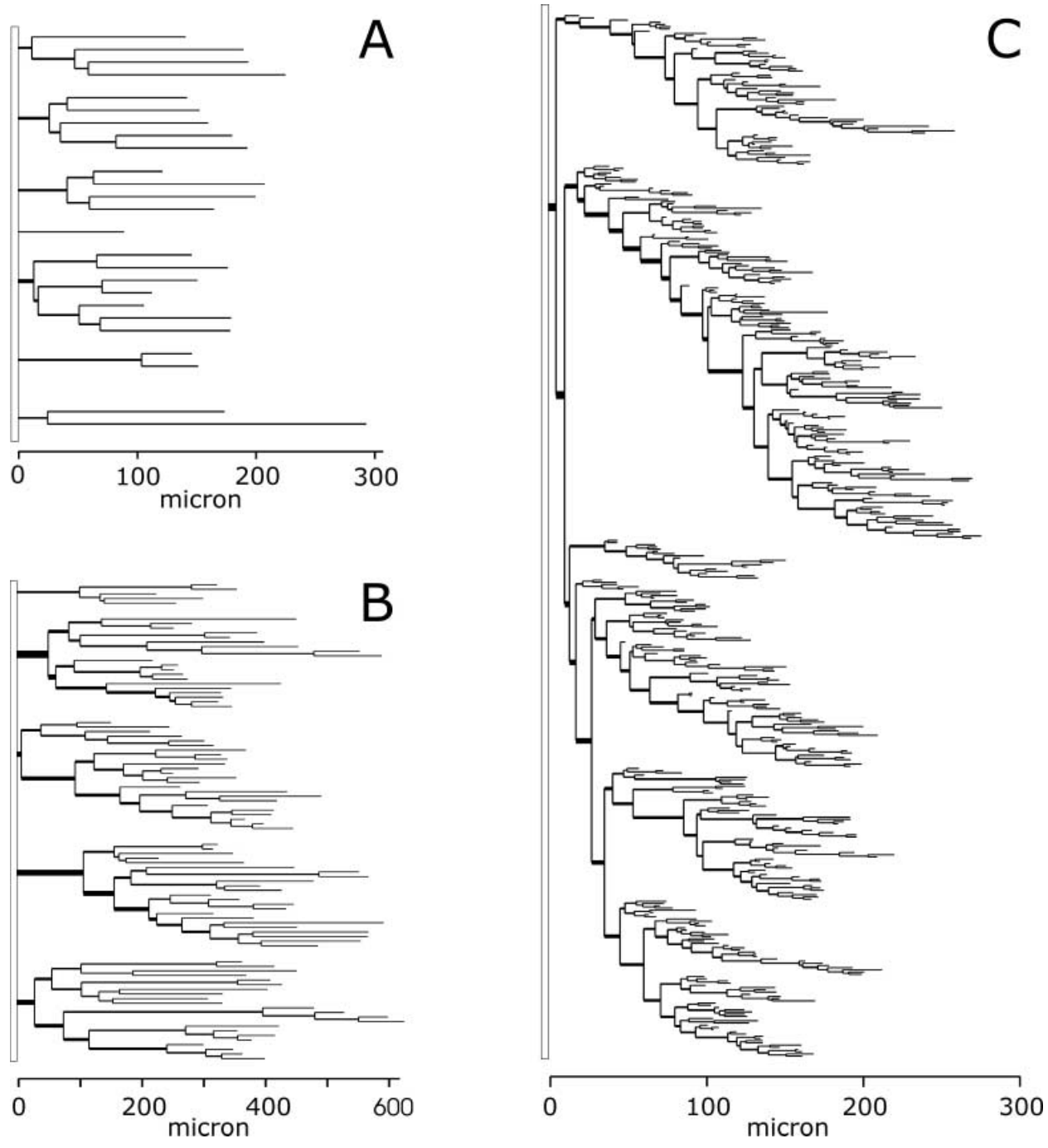

Fig. 2 Random model trees for (**A**) the cell type III parameter set in Table 2 (S1-rat layer 2/3 pyramidal basal dendrites), (**B**) the cell type V parameter set in Table 2 (cat deep layer superior colliculus dendrites), and (**C**) the cell type IV parameter set in Table 2 (guinea pig cerebellar Purkinje cells)

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