# Compartmental Modelling of Developing Neurons

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FINAL NARRATIVE REPORT

## 1 Background and Context

A highly distinctive feature of neurons is their morphology. Neurons exhibit long processes, or neurites, that are fundamental to the formation of the connected networks of neurons that constitute a nervous system. One neurite, the axon, forms the output electrical signalling pathway of a neuron. A typical axon has a main trunk from which shorter side branches, or collaterals, emerge to form points of contact with appropriate target neurons. Axons may be extremely long, up to about one metre in humans, for example. The remaining neurites of a neuron are dendrites, which form complex tree-like structures and are the recipients of most synaptic contacts from the axons of other neurons. Different types of neuron can be distinguished by the morphology of their dendrites, which can be characterized in terms of segment lengths and diameters, the number of terminals (unbranched tips), the number of branch points, and topological factors such as symmetry.

Much of the research in the field of computational neuroscience has been directed at understanding the electrical signalling properties of neurons, with a particular emphasis on the impact of complex neuronal morphology on signal integration. This uses so-called "compartmental" models of neurons which are based upon solving a well understood partial differential equation (PDE) for membrane voltage over space and time, where space is one dimensional along the length of neurites. Powerful numerical techniques for integrating this equation and for coping with the branched structures of neurons have been developed. Sophisticated computer simulation packages, such as NEURON (`www.neuron.yale.edu`) and GENESIS (`www.genesis-sim.org`), make the creation of electrical signalling models a near user-friendly experience. Such packages provide graphical interfaces for both model construction and for the display of results.

However, this research into electrical signalling ignores the fascinating and fundamental problem of how a neuron's complex morphology arises during nervous system development. Understanding of this process is vital if we are to develop therapies for nervous system repair following injury, such as promoting axon regrowth, and the use of stem cells to develop into replacement neurons. Mathematical modelling and numerical simulation are invaluable tools to help us unravel the processes underlying morphological development. The sorts of model that have been investigated so far vary widely and are typically aimed at a particular aspect, such as target finding by axons, rather than describing the entire development of a neuron. Consequently, no uniform mathematical or numerical techniques have yet emerged to lead to the building of the sort of user-friendly software available for modelling electrical signalling. Such software is required for the expansion of this research field.

## 2 Key Advances and Supporting Methodology

This project has had two aims: (1) to formulate new biophysically-based mathematical models of neurite outgrowth, and (2) to develop appropriate numerical techniques for the solution of such models and provide software implementations for computer simulation of them. Both of these aims have been fulfilled, as will be described. Numerical solution of the new models has required novel techniques for the "compartmentalization" of a neuron whose shape is changing with time. Computer simulation software for running these models has been developed in both Matlab and Java to provide cross-platform implementations. This software has been made freely available to the wider research community through the World-Wide Web.

## 2.1 Models of neurite outgrowth

We have formulated a number of new mathematical models that describe the dynamics of cytoskeleton construction underpinning the outgrowth of neurites, based on the known biophysics.

**Morphological development of neurons.** The outgrowth of neurites is a complex process which is far from fully understood. Following its initiation, an individual neurite begins to extend outward from the cell body and may eventually form a branched, tree-like structure. This structure is supported by an internal cytoskeleton built from long polymers, principally of tubulin, known as microtubules. Tubulin oligomers are transferred along the neurite by diffusion and active transport and are used in the assembly of microtubules along the length of the neurite, with the most actively growing microtubules being at the distal end of a neurite. Thus the synthesis and transport of oligomeric tubulin to the distal end of a neurite is a limiting process in neurite outgrowth.

During neurite outgrowth many factors can influence the rate and stability of cytoskeletal construction. The intracellular concentration of free tubulin itself may autoregulate tubulin synthesis. Microtubule-associated proteins (MAPs) control the stability and assembly rates of microtubules in ways which in turn depend on the phosphorylation state of the MAPs. Dephosphorylated MAPs cross-link microtubule bundles and promote microtubule assembly. Phosphorylation of MAPs destroys their cross-linking ability and so destabilises the microtubule bundles, and has been correlated with increased neurite branching. Elongation and branching are influenced by the growth cone at the tip of the neurite. This growth cone senses the external environment and exerts tension on the trailing neurite, which can directly affect microtubule assembly rates and the splitting of microtubule bundles during branch formation. As the neuron as a whole develops, intrinsic and synaptic electrical activity can influence neurite outgrowth through modulation of cytoskeleton construction, possibly through altered intracellular calcium levels setting the phosphorylation states of MAPs.

**Continuum model of neurite elongation.** Firstly, we have formulated a fundamental model of neurite elongation based on cytoskeleton construction using a continuum mechanical approach (Graham et al., 2005; McLean et al., 2004; McLean and Graham, 2004a,b; McLean et al., 2005; McLean and Graham, 2005a,b,c). A partial differential equation (PDE) describes the production, transport and assembly of tubulin dimers into microtubules. This allows explicit calculation of tubulin concentration gradients along the length of the growing neurite. Importantly, the model is amenable to analysis as well as numerical solution. This work has been undertaken in a new collaboration with a mathematician, Douglas McLean (DRM) in the Department of Computing Science and Mathematics at Stirling. DRM formulated and analysed the model. PI Bruce Graham (BPG) undertook numerical simulations of the model.

The model solution, both analytical and numerical, is complicated by the fact that it is of the moving boundary type i.e. the spatial domain changes over time due to changes in the length, $l$. DRM has used analytical techniques, many of which are novel in the context of moving boundary problems, to determine steady-state lengths (McLean and Graham, 2004b; McLean et al., 2005) and their linear and nonlinear stability (McLean and Graham, 2004a, 2005a,b,c). Steady-state analysis has revealed that growth can proceed in three different regimes, as determined by the relative proportions of *construction*, due to the synthesis and active transport of tubulin and its assembly onto microtubules, and *dissipation*, due to tubulin diffusion, degradation and its disassembly from microtubules. If construction dominates dissipation, then large growth results. If dissipation dominates construction then only short lengths are achieved. Moderate growth ensues if construction and dissipation are approximately balanced. The transition between growth regimes is highly nonlinear. This is an important result as it indicates that small changes in, say, tubulin production rate, can result in very large changes in neurite length. Neurite elongation and retraction are a feature of axonal and dendritic development and underpin the ability of a neuron to form appropriate network connections. This work indicates that exquisite control of cytoskeleton construction may play an important role in this process. Further analysis has also revealed that the steady-state length may be linearly unstable, but is always nonlinearly stable. This overall stability may be crucial for neurons to be able to maintain their basic morphology during maturity, while retaining the ability to modulate neurite lengths by small changes in growth parameters, as revealed by the steady-state analysis.

The dynamics of outgrowth have been explored using a numerical solution of the model (Graham et al., 2005; McLean et al., 2004). The stability of outgrowth is confirmed by these numerical simulations. Growth in the large and short regimes is quite damped and is determined by active transport in the large regime and diffusion in the short regime. Oscillations in length can occur in the moderate regime where growth is

affected by both the active transport and diffusion of tubulin. Autoregulation of tubulin production is able to damp out these oscillations. Details of the numerical methods used are given below.

**Basic model of neurite branching.** A major focus has been on the construction of models that include neurite branching as well as elongation. In addition to considering the rate of neurite elongation due to microtubule assembly, these models also consider the stability of the resultant microtubule bundles as determining the likelihood for a neurite tip to bifurcate.

BPG, working with project partner Arjen van Ooyen (AvO), has developed a basic model that considers whether the production and transport of an unspecified branch-determining substance imparts constraints on branching (Graham and van Ooyen, 2004). The model has been defined as a set of coupled ordinary differential equations (ODEs) that each describe the transport of the branch-determining substance along one unbranched segement of the developing tree. The concentration of the substance at the tip of each terminal segment determines the probability that the terminal would split to form two daughter branches.

Statistical models of dendrite formation indicate that the probability of a neurite bifurcating is modulated as the tree grows and is a function of the number of terminals in the tree and the number of branch points between a particular terminal and the cell body (centrifugal order) (van Pelt and Uylings, 1999). Our basic model shows that such modulation of branching probability can arise from variations in the availability of a branch-determining substance at each terminal due to the diffusion and active transport of that substance from its site of production in the cell body. Such a substance can plausibly be identified as tubulin, or possibly a microtubule-associated protein, such as MAP2, that influences the stability of microtubule bundles in dendrites. Thus the major outcome of this model is that the branched tree structure of the developing neurite can itself influence its further development by either promoting or inhibiting the transport of growth-determining substances to individual neurite tips. Depending on how segment diameters develop, a more highly branched subtree may sequester proportionally more or less of the substance. This process can produce dendritic structures as found in different types of real neurons with tight matches to the topological statistics (Graham and van Ooyen, 2004; van Pelt et al., 2003).

**Biophysical model of neurite elongation and branching.** The work of the PhD student Gregor Kiddie (GAK), employed by the project studentship provided by this grant, has been to formulate a much more sophisticated model, based on the work of Hely (Hely et al., 2001), that describes both the elongation rate and the branching rate of a neurite tip explicitly as functions of tubulin and MAP2 concentrations at the tip (Kiddie et al., 2004a,b, 2005a,b,c). The aim is to explore whether both the segment length distributions and branching structures found in real dendrites may arise from the constraints on cytoskeleton construction.

In this model, both tubulin and MAP2 are assumed to be synthesised in the cell body and transported by diffusion and active transport along the growing dendrites. At a terminal, the amount of free tubulin and MAP2, and the phosphorylation state of MAP2 determine the elongation and branching rates. Dephosphorylated MAP2 acts as a cross-linker between microtubules, stabilising the bundles and promoting microtubule assembly. Phosphorylated MAP2, on the other hand, loses its cross-linking ability and destabilises the microtubule bundles, thus increasing the likelihood of a bifurcation event. (De)phosphorylation of MAP2 is a Michaelis-Menten function of the calcium concentration, with small changes in calcium possibly resulting in a large shift in the balance between phosphorylated and dephosphorylated MAP2. Calcium entry is assumed along the length of the dendrite (putatively through voltage-gated channels). The biochemical (de)phosphorylation pathways, involving at least CaMKII and calcineurin, are not explicitly modelled.

This complex model of elongation and branching has been implemented as a coupled system of ODEs, with their numerical solution following a "compartmental" approach developed by BPG (Graham and van Ooyen, 2001), as has been used for solving the voltage equation for simulating electrical activity. Details of this approach are given below.

This model has been used to try to gain insight into the fundamental mechanisms that control the development of the characteristic dendritic morphologies of different neuronal types. Results to date indicate that the precise relationship between calcium concentration and MAP2 phosphorylation can lead to the development of specific forms of tree. These forms range from trees with short terminal segments and long intermediate segments, to relatively uniform segment lengths, to long terminals with short intermediates. This spans the space of real neuronal dendritic morphologies.

## 2.2 Numerical approaches

Our ODE and PDE models of neurite outgrowth describe the intracellular environment as one-dimensional. Numerical solution of these models involves solving for chemical concentrations at particular points along the

length of a neurite. The spatial discretization is complicated by the fact that the cell morphology changes over time, necessitating continual respecification of the discretization. We have employed two different ways of tackling this. Example Matlab and Java code for our solutions has been made available on the World Wide Web (from BPG's website and on the ModelDB database maintained by Yale University Medical School at http://senselab.med.yale.edu/senselab/modeldb) for free use by other researchers.

**Fixed compartment number.** Numerical solution of the PDE model of neurite elongation employed a spatial domain which is discretized into a fixed number of grid points (or compartments), with integration being carried out on a spatially-transformed domain in which $x$ lies only in 0 to 1 (Graham et al., 2005). Transforming back into the real length domain results in the grid points growing further apart as the length increases. A second-order accurate finite difference scheme following the Crank-Nicholson approach was used. For numerical stability it is important that the number of grid points is sufficient for the discretization of the maximum expected length. This may be an unnecessarily large number for the early stages of growth when the length is short.

The fact that the compartments are not positionally static may also be problematic for modelling particular situations. For example, modelling synapse formation during neurite outgrowth and the subsequent response of the neuron to synaptic input requires that the exact spatial location of the synapse be stationary.

**Fixed compartment size.** An alternative approach was taken for the solution of the ODE "compartmental" models of neurite elongation and branching (Kiddie et al., 2005a). In this approach, a neurite is divided into a number of short compartments of mostly equal length, with chemical concentrations being calculated for the volume of each compartment. Chemicals move between compartments due to bulk diffusion and active transport. As a neurite elongates, new compartments are added when needed. New compartments are also created when a branching event occurs. Various strategies for when and where new compartments are added have been investigated (Graham and van Ooyen, 2001).

In this scheme most discrete points at which chemical concentrations are calculated are positionally static. This may be advantageous for modelling neurite interaction with the external environment, including contact and synapse formation with other neurites. However, specifying a single compartment length is inefficient. A size that is small enough for accurate calculation in short neurite branches may lead to older, longer branches being described by an unnecessarily large number of compartments with a resultant high computational load for numerical simulation. We have also so far relied on a numerical solution based on simple forward Euler integration. This makes adding compartments very easy but requires excessively small time steps for numerical accuracy. A second-order accurate implicit integration scheme is to be preferred, but would require more work in redefining coefficient matrices whenever the compartment number changes.

Both this fixed compartment size and the fixed compartment number approaches to spatial discretization thus are not optimal in all situations, but both provide practical solutions. Further work is required to reconcile these two approaches and to perhaps formulate adaptive discretization schemes that choose the optimum method for different situations.

# 3 Project Plan Review

The original project plan included two themes: (1) the development of computer simulation tools for modelling neuronal development, and (2) the formulation of biophysically-based mathematical models of the development of neuronal morphology. Both of these themes have been pursued in accord with the plan, with results detailed above.

Progress in both themes was enhanced by a new collaboration with mathematician Douglas McLean (DRM). Drs McLean and Graham employed two summer students, one in 2004 and another in 2005, to contribute to this work. The work of the 2004 student, Karen Lauchlan, has been included in two journal publications (Graham et al., 2005; McLean et al., 2005). Conference presentations have been made by DRM at Computational Neuroscience, Alicante, July 2003; First Canada-France Meeting of Mathematical Sciences, Toulouse, July 2004; and the European Conference on Mathematical and Theoretical Biology, Dresden, July 2005.

PhD student Gregor Kiddie (GAK), employed on this grant's project studentship, concentrated largely on the formulation of biophysically-based models for neurite elongation and branching. He has developed computer simulation software in both Matlab and Java for the deployment of these models. The Java code is now stable and simulation runs of the models are continuing. Kiddie has also begun the write-up of his dissertation. He switched to part-time study at the end of the grant period as he is now in full-time

employment in industry. It is still anticipated that he will submit within the University of Stirling time-limit of August 31st, 2007 (equivalent to 4 years full-time study). Throughout the project his progress was monitored by six-monthly reporting, with the student submitting an annual detailed report of progress which was subject to a viva in front of a panel of three examiners: Drs Graham and McLean and Prof. Smith from Computing Science at Stirling. Review meetings with project partner Dr van Ooyen took place in July 2003, March and August 2004, and June 2005.

GAK attended an EPSRC Graduate School in 2005. He also obtained specialist training at the 23rd International Summer School of Brain Research in Amsterdam, August 2003 and at the Edinburgh Neuroinformatics Simulation Tools Summer School (part funded by EPSRC) in August 2004. He has presented posters of his work at the Brain Research Summer School and at the Forum of European Neuroscience meeting in Lisbon, July 2004. He has given talks on his work at the Biologically-inspired Computing Systems conference, Stirling, September 2004, the WiR Workshop on Data-driven Modelling and Computation in Neuroscience, Germany, May 2005 and the Computational Neuroscience conference, USA, July 2005.

Different numerical techniques have been specified for the simulation of our neuronal development models. Full details of a new scheme have been published (Graham et al., 2005) and two overviews of the techniques, along with suggestions for what a complete "neuronal development simulator" might comprise, have also been published (Graham and van Ooyen, 2005; Kiddie et al., 2005a). Currently our simulation techniques have been deployed in custom Matlab and Java computer code, which has been made freely available to the research community via the world-wide web. Fundamental work is still required on the numerical techniques. Consequently they have not been deployed within existing simulation software such as NEURON, though this remains a future possibility.

# 4 Research Impact and Benefits to Society

This project has involved fundamental research that is of most immediate importance to those using mathematical models and computer simulations to study the operation and development of the nervous system. The emphasis has been not just on results from particular models, but in also creating numerical techniques and associated computer software that are useable by the computational research community. The techniques have been published and the software is freely available on the Web, as detailed above.

The work has been recognised by the emerging Systems Biology field. Invited talks by BPG have been given at the SYMBIONIC "Computational Systems Biology of the Neuronal Cell" school in Trieste, December 2004 and at a follow-up workshop at the end of the EU Systems Biology school in Gosau, Austria, March 2005.

# 5 Explanation of Expenditure

Expenditure was largely as budgeted and specified in the original grant proposal. Continuing reduction in the price of computing equipment enabled two dual Xeon processor PC workstations to be purchased, as well as a powerful laptop PC. Reductions in airfares aided the travel budget. In addition, several collaborative meetings with Dr van Ooyen, Amsterdam, took place in conjunction with other events, reducing the expected travel expenditure. Due to increases in journal prices it was decided the purchase of background texts in experimental and computational neuroscience was better value and of more use, particular to the PhD student (GAK), than the purchase of a subscription to the Journal of Computational Neuroscience, as originally intended with the Consumables budget.

# 6 Further Research or Dissemination Activities

The PhD work of GAK remains to be written as a dissertation. It is anticipated that at least one journal paper describing the model and key results will also result.

Further work on numerical techniques and associated computer software will continue, carried out by BPG in collaboration with DRM and AvO. An outline of what we are aiming for is in press (Graham and van Ooyen, 2005). Additional research funds will likely be sought for this.

DRM and BPG are seeking EPSRC grant funds to continue the analytical work on the continuum model of neurite elongation, looking particularly at extending the model to cover neurite branching as well (proposal in preparation). An earlier proposal in this area to EPSRC (December 2003) was unsuccessful.

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