

MODELING THE ASSEMBLY OF THE AXONAL CYTOSKELETON DURING STRETCH INDUCED GROWTH: OPTIMIZING CONDITIONS FOR GROWTH

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INTRODUCTION

Strategies for repairing the injured nervous system span technologies that include targeted drug treatments to inhibit neuronal degeneration, transplants to replace or sustain cell function, and biomaterials implanted to promote axonal growth and repair. We presented recently a new strategy to build tissue transplants for the CNS (Smith et al., 2001) that uses the principle of mechanically induced neurite growth, a concept first demonstrated by Bray (1984) and later studied in more detail by Lamoreux et al. (1989) and Zheng et al. (1991) in single neurites. However, we extended this concept by developing a technique to simultaneously grow several thousand axons in vitro. We did not focus on inducing growth through the modulation of growth cone dynamics; rather, we grow these axons once they have synaptically connected with an adjacent neuronal population.

In this report, we model the assembly of the axonal cytoskeleton during controlled axonal growth. We include the production, polymerization, and transcriptional control of neurofilament protein as the limiting factor for maintaining axonal caliber during growth. We estimate the factors that limit growth rate for axons using this technique, and propose combination of growth rates and steps that will allow the long term growth of cultured axons in vitro.

MATERIALS AND METHODS

Neurofilaments are heteromeric polymers that occur in three distinct forms in vitro according to their approximate molecular weight – proteins of either 68 kDa (NF-L), 150 kDa (NF-M), or 200 kDa (NF-H) in weight. From recent in vitro studies on neurofilament transport, it is now widely believed that the neurofilaments are polymerized from monomeric subunits in the soma, transported down the microtubule network in the axon in the filamentous form, and actively degraded into the monomeric subunits at the axon terminal. Along the length of the axon, polymerized neurofilaments can be degraded by proteases

within the axoplasm, and the monomer subunits can also self-assemble back into the filamentous form within the axoplasm.

Within a control volume in the axon, there are four separate pools of neurofilament to consider – the subunit pool, the polymerized fraction that is stable (C_S), or within the cytoskeletal framework, the polymerized fraction that is trafficked via fast anterograde axonal transport (C_A), and the polymerized fraction that is retrogradely transported towards the soma (C_R). Existing data from photobleaching studies indicates that the subunit pool (C_M) is a small proportion of the pool in the axon, estimated at less than 5%. Additional and more recent studies have shown that there is a dynamic equilibrium among the three polymerized pool fractions. The equilibrium among the polymerized components can be approximated using simple first order kinetics models to describe the switching between states:

$$\frac{dc_{S,i}}{dt} = -(K_{SA} + K_{SR}) \cdot c_{S,i} + K_{AS} \cdot c_{A,i} + K_{RS} \cdot c_{R,i}$$

$$\frac{dc_{A,i}}{dt} = -(K_{AS} + K_{AR}) \cdot c_{A,i} + K_{SA} \cdot c_{S,i} + K_{RA} \cdot c_{R,i}$$

$$\frac{dc_{R,i}}{dt} = -(K_{RA} + K_{RS}) \cdot c_{R,i} + K_{AR} \cdot c_{A,i} + K_{SR} \cdot c_{S,i}$$

where K_{xy} denotes the rate constant from state x to state y. Within the control volume, we assume that the degradation and self-assembly can be approximated with first order kinetics (K_{pol} , K_{diss}), and that the instantaneous transport velocities in the anterograde (V_A) and retrograde (V_R) direction are distinct. If new neurofilaments are assembled, we assume that they are distributed equally among the three filamentous species according to their respective equilibrium fractions.

Of the four species, only the monomeric form can freely diffuse through the axonal compartment; the remaining species are either part of the stable network or are transported exclusively with molecular motors. The resulting equations for the four separate species in the axon control volume are:

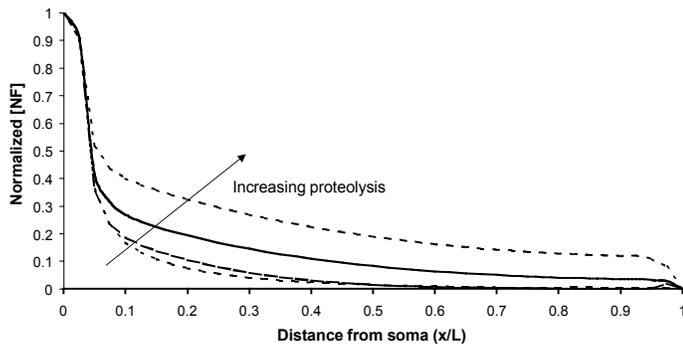
$$\begin{aligned} \frac{dc_{A,i}}{dt} &= -(K_{AR} + K_{AS}) \cdot c_{A,i} + K_{RA} \cdot c_{R,i} + K_{SA} c_{S,i} + \\ &\quad \frac{V_A}{2\Delta x} (c_{A,i-1} - c_{A,i+1}) + K_{pol} c_{M,i} \cdot \chi_A - K_{dis} c_{A,i} \\ \frac{dc_{R,i}}{dt} &= K_{AR} \cdot c_{A,i} - (K_{RA} + K_{RS}) \cdot c_{R,i} + K_{SR} c_{S,i} + \\ &\quad \frac{V_R}{2\Delta x} (c_{R,i+1} - c_{R,i-1}) + K_{pol} c_{M,i} \cdot \chi_R - K_{dis} c_{R,i} \\ \frac{dc_{S,i}}{dt} &= K_{AS} \cdot c_{A,i} + K_{RS} \cdot c_{R,i} - (K_{SA} + K_{SR}) \cdot c_{S,i} + \\ &\quad K_{pol} c_{M,i} \cdot \chi_S - K_{dis} c_{S,i} \\ \frac{dc_{M,i}}{dt} &= \frac{D}{\Delta x^2} (c_{M,i-1} - 2 \cdot c_{M,i} + c_{M,i+1}) - K_{pol} c_{M,i} \cdot \chi_A + \\ &\quad K_{dis} c_{A,i} - K_{prot} c_{M,i} \end{aligned}$$

where $i+1$ and $i-1$ denote the concentrations in the distal and proximal compartments, respectively, and Δx is the incremental length of the control volume compartment studied. At the axon terminal, the filamentous forms of the neurofilaments are actively degraded, and there is no net flux at the terminal for all species. In the soma, there is only an influx of retrogradely transported filaments from the axon hillock, an anterograde transport out of the compartment, and a free diffusion of monomer.

With these boundary conditions, the axon is discretized along its length to develop a system of equations that describe the dynamics of the neurofilament transport along the length. The resulting system of first order differential equations is solved over time using a fourth order Runge-Kutta scheme, and adaptive stepsize control is used to advance the solution quickly over slowly varying solution domains. The spatial mesh is reduced to a size to allow for sufficient spatial coverage over the range of simulations used, and the incremental length used is held constant for all simulations.

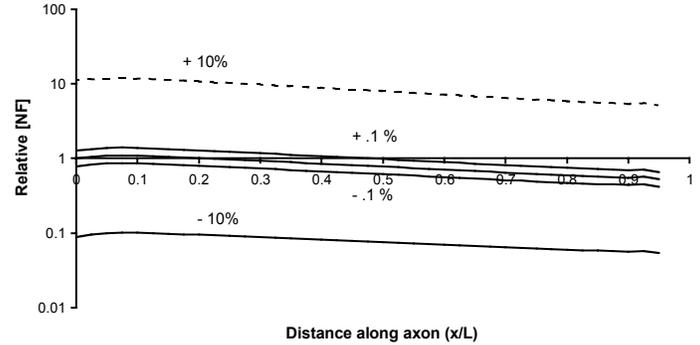
MODELING RESULTS AND DISCUSSION

Using estimates of the proteolytic activity for major proteases within the axon (e.g., calpains) and approximations for the assembly/disassembly kinetics and the switching between states, the resulting neurofilament concentration is predicted to decrease with increasing distance from the soma (Figure 1). The relative density of the neurofilament architecture is dependent on the proteolytic activity assigned to the axon, with a net decrease in the architecture occurring with increases in the activity of the proteases.

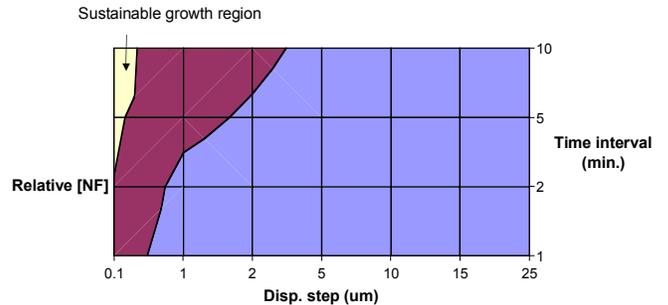


Simulations results show that deficits will begin to appear in the neurofilament architecture under growth conditions unless new

neurofilament is added to the axonal compartment (Figure 2). With the volume of the axon increasing under continuous mechanical tension, more polymerized neurofilament is needed to maintain the density along the axon length. Deficits, defined as the relative neurofilament concentration falling below a normalized value of 1.0, appear in more distal segments of the axon. Increasing the production of neurofilament in the soma will mitigate the deficits along the distal segments of the axon; if somatic production is increased sufficiently, no deficits appear along the axon length (dashed line, Figure 2).



Assuming a nominal increase in the neurofilament production in the soma, optimal combinations of the displacement amount and interval between displacement steps can be calculated, providing an estimate of the growth conditions that will allow the sustained growth of axons in vitro (Figure 3). These estimates are within the measures that have been measured experimentally in cultured NT2 cells. More recent work with other neural cultures shows a range in the sustainable growth rates, pointing to possible changes in the control of neurofilament production among these cell types.



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