Systems biochemical model of neurodynamics

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Abstract

Neural growth is directed by extracellular signal molecules that can either deter or encourage growth. Those molecules cause internal signal response that effects gene expression and ultimately cell fate. These dynamic signal molecules can be quantified at the systems scale with the nascent fields of proteomics and metabolomics. Molecular-scale "omics" measures can be combined with cell-level phenotypic and fluorescence microscopy methods as a collective input to theoretical models, which are used to validate assumptions and generate testable hypothesis. A complete model of neurodynamic growth, once complete, promises to expedite generation of therapeutic targets and cures for neurological diseases. Here I describe the implementation of a model that allows scalable input from systems signal molecule measures and microscopy data. The package written in Java is named "Axon." I envision this package will in the future accept real-time imaging data, histological data, and omic-scale molecular measures independently. This provides a publicly available framework that bridges computational and experimental biology.

1 Introduction

1.1 Extracellular signaling

Several extracellular molecules are known to interact with growing neural tissue. Among signal molecules that are detrimental to growth, thrombin is known to deter neuron growth by cleavage of extracellular membrane receptors [1,6]. Thrombin is a unique protein in that it both prevents and promotes blood clotting in a manner that depends on other protein and small molecule regulators through an allosteric mechanism. This detrimental quality of thrombin on nerve cell growth seems logical since the presence of blood from injury would certainly require a local evacuation to salvage living tissue followed by repair and recolonization. Individuals who's neurons do not respond to such extracellular signals would almost certainly have aberrant signaling in as a result of the unique interactions of their receptors.

1.2 Intracellular signaling

The response to extracellular signals of a nerve cell depends upon intracellular signals. Several protein factors are known to be synthesized and degraded locally in extending axons and dendrites [2]. Signals are also know to be transported towards the cell body at an alarming rate (100-200 mm/day) [7]. The mechanisms of transport along microtubules and actin filaments is a topic of great interest. In fact, the transport of organelles such as mitochondria and ribosomes toward the growth cone is required for some responses to extracellular signals [7].
Some viral particles have evolved to exploit these transport mechanisms to allow specific targeting to neural cell types [3]. Because of this, an understanding of the molecular level players would

facilitate targeted drug design in a similar fashion. In fact, the transport process itself is still poorly understood but will benefit from quantitative proteomics and quantitative metabolomics.

1.3 Model description and purpose

A powerful method for neurological model input is florescence microscopy, because of its selectivity and sensitivity towards analytes such as Ca^{++} and protein markers. I propose here an extension of this traditional method to include post imaging measurement of protein expression. Mass spectrometry based proteomics has rapidly mature and routinely allows quantification of high abundance proteins from cell lysates[6]. Therefore, we can correlate cell-level growth observations with molecular-level proteome measurement to enhance model accuracy. The use of molecular scale information in neuron modeling is not required, as these parameters can be lumped into one or several variables without actually understanding individual protein contributions to the observations. Although these models can accurately recapitulate the microscopy data, the inclusion of molecular data allows the model to be used for development of therapeutic targets. The vast majority of effective and safe drugs target membrane receptors. Neural tissue allows the unique mechanisms of synaptic communication and intracellular transport along the neurite element.

2 Methods

2.1 Qualitative model description

For proof of principle, we consider a simple system with one neuron growth source and two responding neurons with extending dendrites. This particular simulation design was designed with careful attention to experimental constraints resulting from neural culture, microscopy and subsequent proteome measurement. Neurites from the two cells should be allowed to grow inward toward the signal molecule diffusion gradient, and should respond to multiple competing factors as would be the case in vivo (figure 1). We also consider the relevant intracellular molecules including tubulin, which serve as scaffolds in neurons similar to the way railroads serve as static pathways for transport. Most importantly, the extracellular and intracellular molecules interact to ultimately produce the neurons behavior.

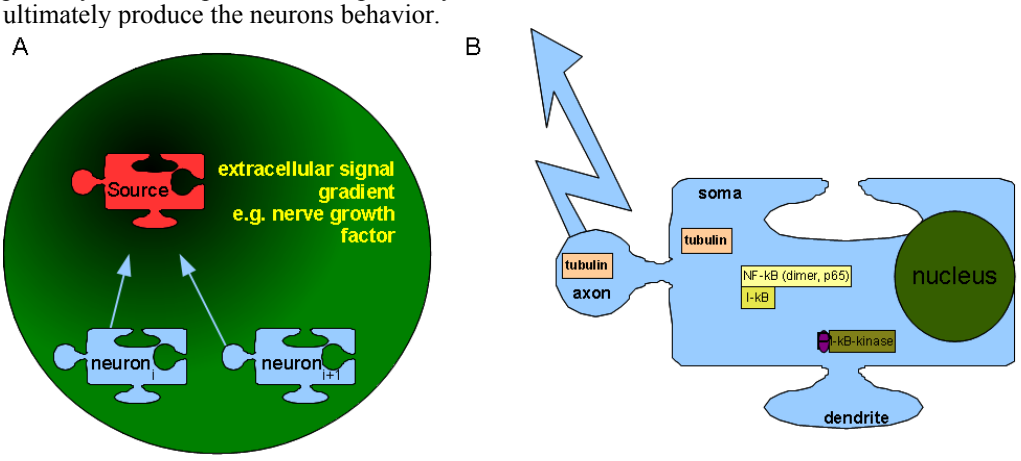

Figure 1: Cartoon of relevant model components.

(A) Neuron-level schematic of the theoretical experiment showing a single, inhibitory source neuron growing on a petri dish and secreting a signal molecule into the media that attracts axon growth. The three neuron system allows competition based simulations where similar parameters can be assigned to each of the blue neurons to compare effects. Also note that this system could easily be replicated in the lab to provide model refinement parameters.

(B) Cell-level schematic of the theoretical experiment showing the included cell elements soma, axon, and dendrite, as well as the intracellular molecule tubulin. NF-kB related signal molecules are included, but were not yet implemented due to time constraints. Not pictured molecules include: glutamate, thrombin, caveolin, and the ambiguous class “food” to allow resource reqs.

2.2 Simulations

The model implementation was written in java using the CX3D library [Zubler, F. and Douglas, R.]. The CX3D library has several tools already written and is designed to be highly extensible and portable. Additional value in CX3D comes from the ability to export simulated architectures to NeuroML for further processing and/or simulation with NEURON. CX3D also has a long list of tutorial simulations that are included to assist the user in understanding the framework. First, several classes were written to recapitulate the model shown in figure 1, and then multiple runs were performed with various parameters of interest. The package together was named "axon." and the code is appended to the end of this document. Those wishing to run the code should add the cx3d library as a reference to my package before compilation. The input parameters for the model were chosen based on the literature here, however, the code was written to allow for experimentally measured inputs.

2.3 Quantitative description

Several parameters quantitatively effect the model outcome. First, the axon direction for each time step is determined by the normalized average of the molecule gradient with the previous direction and random vector. This appears to address a central issue proposed in [] Additionally, the internal concentrations of both tubulin and caveolin proteins are secreted at a constant rate, k, according to the following equations:

$$[tubulin]_{t,\,soma} = k * [tubulin]_{(t-1),\,soma}$$

Where k, in the case of the model implemented here, is equal to:

$$k = 1 + \frac{60}{([tubulin]_{(i-1),\,soma})}$$

In this case resulting in a linear increase of the molecule.

3 Results

Several classes in java were written to allow simulation of the desired neurodynamics (table 1). The goal of the package was to maintain flexibility and scalability in The class allows each neurite to respond to specific substances either positively or negatively, and specifies branching behavior in response to that substance. The class also incorporates the effects in the intracellular concentration of tubulin, which is generated mostly in the soma with this model. I also changed the functionality of the synapse formation.

3.1.2 MyNetwork1

The first model I wrote was simplified to include only two soma with a single neurite each. I placed an artificial gradient between them and set one of the neurites to be attracted to it. I wrote a module for chemical repulsion and made the neurites secrete each a different signal molecule for the other neurite to be attracted to (figure 2A). The behavior is evident as the top neurite appears to follow the other neurite after they come in close proximity.

3.1.3 MyNetwork2

Next, I added the ability of the neurites to attempt synapse formation every 100 time steps, and made the chemical effect class more flexible by allowing the input of substance ID, branching propensity, and positive or negative direction modificiation (NeuriteChemoEffect.java). I initially had the neurites attempt synapse formation every time step, but this drastically slowed the simulation. Every 100 time steps accomplished the effect without drastically increasing simulation time.

3.1.3 MyNetwork2

This network adds terms for speed and direction of growth dependent on intracellular and extracellular signals. This model places an arbitrary number of inhibitory and excitatory neurons with user specified locations and numbers of neurite elements. Each neuron is given one axon (output) and one dendrite (input) and additional neurites are of undefined type.

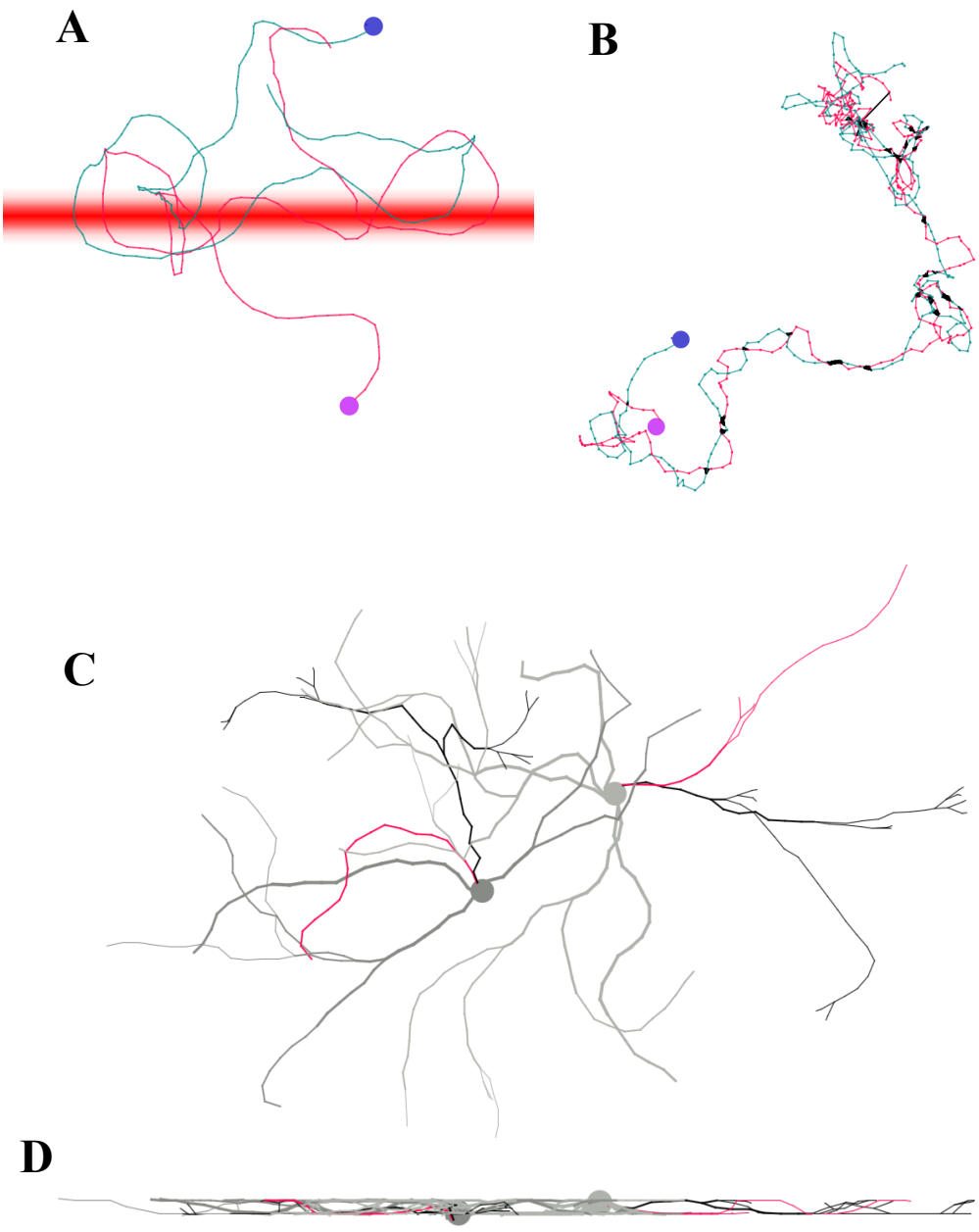

Figure 2: Sequential implementation of model features

(A) An example output from MyNetwork1.java simulation of two neurons, each with a single, non-branching neurite. The red neurite from the bottom soma is attracted to the red substance, which is also secreted by the teal neurite from the top soma. The teal neurite is not attracted to the red substance, but is attracted to a substance secreted by the pink neurite. **(B)** An example output from the simulation class: MyNetwork2.java with the same parameters, but without the artificial gradient of the red substance. This model adds the ability for the neurites to form synapses, which are represented here as black bars connecting the neurites. The synapse formation depends on the physical proximity and the presence of boutons and spines along the neurite (not pictured). **(C)** An example output from the simulation MyThirdNetwork.java showing two neurons, one excitatory and the other inhibitory, each with three types of neurites coded by color. This model incorporates scalable inputs of signal molecules, both intracellular and extracellular, which can be used to effect growth and synapse formation. **(D)** The output from (C) rotated on the X-Z axis to show the tissue culture inspired constraints on the neurite growth.

3.1.4 WorkingModel

The final model incorporates all features of the first three in a very scalable manner. The type of cell (excitatory or inhibitory for NeuroML export), number of cells, neurites per cell, substance responses and substance productions are all scalable inputs. Figure 4 shows an example of the qualitative and quantitative output generated from this interdisciplinary and multiscale-biology model. As an example application of this models utility, I ran several simulations varying the production rates of tubulin and counting the number of synapses formed between the two cells. However, I was unable to compile this data in time, but I hope to include this in a peer-reviewed publication in the near future.

Table 1: Table of scalable model input parameters

Object class	**Optional settings**
NeuriteElement*	• Dendrite (input terminal), axon (output terminal), or other • Chemical effectors and how they modify growth direction and rate
Soma* (Cell Body)	• Type: excitatory or inhibitory • Number and locations • number and positions on neurites [0<position<2π]
Master Movement Module	• Replaces class "NeuriteChemoEffect" for comprehensive movement decisions • Object direction and speed dependent on the sum of intracellular and extracellular effectors
Internal secreter	• Allows secretion of internal molecules
External secreter	• Allows external molecule modification
MyPetriCells	• Creates a "petridish" of user defined number of neurons with variable neurites that can be either soma, dendrite, or other, all with definable molecular dependencies.

**This class is provided with the distribution of CX3D.*

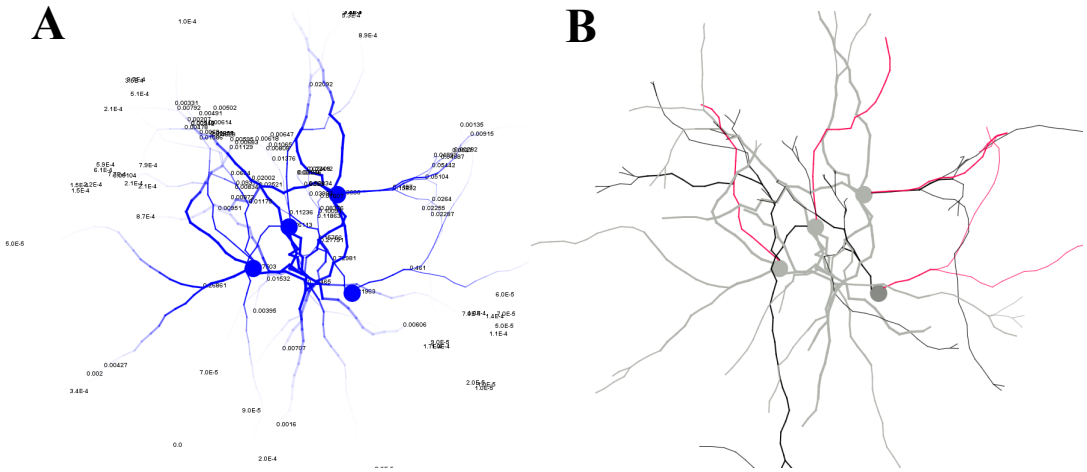

Figure 4: Intracellular quantities of caveolin and the qualitative neural tissue

(A) The plotted intracellular quantities of caveolin, which is used to determine neurite branching propensity. (B) The qualitative image of the same simulation. Here the red neurite is the axon, the dendrite is the black neurite, and the gray element is an undefined neurite element. The model shows that dendrites do not contain caveolin in this simulation, however this is not biologically accurate and is included only for the purpose of illustrating the differential dependencies the package allows.

4 Conclusions and direction

This work lays the groundwork for a comprehensive model of neurodynamics during growth and development of axons. This represents the first environment the author is aware of that aims to bridge theory with experimental input.

3.1.4 Future Directions

One addition I would make to the movement engine is to add more molecules classes to increase accuracy and flexibility of the molecules. The model currently allows for only attractants, repellants, and internal molecules. Four classes of signal molecule objects will be added: 1) Membrane-type internal signals to act as receptors; 2) Kinase-type internal signals to act as internal modifiers of signals; 3) Phosphatase-type internal signals to remove phosphorylations; 4) Nuclear-type internal signals to act on internal secretion of signal molecules. Eventually, classes for all types of Gene Ontology (GO) classifiers will be incorporated.

5 Acknowledgments and reflections

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My goal in taking this course was to learn about neuroscience and programming. When I started, I had about 6 months of experience actually writing [R] code. Now, as I complete this project I learned to write code in both python and java. The neurobiology was also very intriguing and new to me. I was blown away by the mini-symposium on brain-machine interfaces (BMI). My favorite talk was by Dr. Todd Coleman. I have endless ideas about where to incorporate my biochemical understanding with the study of neural systems at the molecular-level; I look forward to collaborating on these topics in the near future. This course far exceeded my expectations and I will emphatically recommend it to my peers.

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