Overview of Techniques for Various Aspects of the C. elegans Modeling Project

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version 0.9, compiled 21:27, Tuesday 5th October, 2010

Introduction

A project as ambitious as realistically emulating the nervous system of an entire organism necessarily consists of many parts and stages. In addition, in our project, there are multiple promising technologies that can serve each of these. In this document, I've identified four main phases, which correspond roughly to the phases of the scientific method—observation, the collection of data about what is happening in the neurons; modeling, the synthesis of this data into predictive models of neuronal function; stimulation, the perturbation of the nervous system so as to collect more nuanced data about its functional relationships; and finally, verification, the techniques for determining the accuracy or fitness of the models produced by the coaction of the other parts of the project.

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1 Observation

Goal: Collect neural activation data

1.1 Optics

It has been suggested that a spinning-disk confocal microscope is the best platform for imaging flourescence in individual *C. elegans* cells. Other possibilities include two-photon microscopy and scanning-laser confocal microscopy. As I know rather little about optics and understand only the basic principles of operation of these devices, I leave it to others—or better, to empirical trial—to determine which of these is the most promising.

1.2 Sensor Molecules

Many calcium- and voltage-sensitive dyes are commonly referred to in the literature, including:

- **RH-155** *(voltage-sensitive)*
- **RH-414** *(voltage-sensitive)*
- **RH-482** *(voltage-sensitive)*
- **Cameleon** *(calcium-sensitive, ratiometric, commonly used in worms)*
- **GCaMP2** *(calcium-sensitive)*
- **GCaMP3** *(calcium-sensitive, 3 times brighter than GCaMP2)*
- **GCaMP5** *(calcium-sensitive, not shown to work in worms)*

For the time being, GCaMP3 is being considered as the leading candidate, but this may change due to new information or as novel molecules are introduced.[1]

1.3 Signal Separation

As nearly all of the data collected by these means will consist of images, it will be necessary to apply some computer vision techniques, at least in the initial stages of analysis. It may also be necessary to apply some of the genetic approaches discussed in section 3.3.

1.3.1 Straightening

The first step to processing these images will be to straighten and register the posture of each animal on a common anterior/posterior/left/right/dorsal/ventral coordinate system. Parts of this can be done manually, but work is underway to implement the algorithm of [**?**] and apply it to the preliminary data of October 6.

1.3.2 Neural Labeling

In addition, it would be desirable to separate the time-varying signals of each neuron algorithmically. However, a full labeling of neurons may prove intractable, and depending on the performance of the straightening algorithm, it may be possible to run modeling techniques directly on the straightened and registered image data. Intermediate approaches are also possible.

[1] Note: as of this writing (October 5), we are using GFP in place of a voltage- or calcium- sensitive dye, because such transgenic constructs are already available, and as a test for the optics and signal separation areas of this phase.

2 Modeling

Goal: Estimate functional relationships from experimental data

This is probably the least developed section in my current thinking. A great deal of discussion and learning will need to take place before this part of the project is well characterized. Some initial scattered thoughts are represented below.

2.1 Correlation Matrix

A simple way to begin, given time-series data of multiple signals from separated neurons, is to compute the correlations of each signal with each other signal, with some selection of time delays. This would elucidate the simplest of functional relationships (e.g. calcium rising in neuron 1 causes calcium to rise in neuron 2 100ms later). It would even be possible to represent the entire nervous system by a Markov model given this matrix of correlations. Although I doubt this would produce very biologically meaningful results, it is a fairly simple first step to try it and see what happens—it might work better than I expect.

2.2 Kernel Methods

However, a correlation matrix would be a very basic analysis of the data. Much more mathematical investigation is needed, but preliminary research suggests that kernel methods are most appropriate, due to their resistance to curse of dimensionality, in addition to the lack of necessity to choose an *a priori* physical model.

2.3 Control Theory

It may also be possible to use some modeling techniques from control theory in real time with an optical stimulation setup as described in section 3 in order to more accurately determine functional relationships.

3 Stimulation

Goal: Control for certain neural variables (while also collecting data)

Beyond inferring functional relationships by examining time-series data of calcium activation under normal *C. elegans* behavior, we can gather more information by controlling for some of the variables; that is, by using optogenetics to directly transiently stimulate or inhibit the activity of specific neurons, and observe the effects on the rest of the nervous system.

3.1 Opsins

Optogenetics is the expression of certain opsin proteins in neurons for the purpose of optically controlling neural activity. The Optogenetics Resource Center lists the following opsins:

- **VChR1**, *Volvox* Channelrhodopsin-1 *(excitatory; older, obsoleted by ChR2)*
- **NpHR**, Halorhodopsin *(inhibitory; known to work in* C. elegans, *silences around 100 pA)*
- **ChR2**, Channelrhodopsin-2 *(excitatory; known to work in* C. elegans*)*
- **hChR2**, humanized ChR2 *(excitatory; bistable)*
- **ChETA**, engineered ChR2 *(excitatory; very fast)*

- **eNpHR3.0**, third-generation engineered NpHR *(inhibitory; silences around 900 pA)*

In addition, at least two recent rhodopsins have been developed by the Boyden group:

- **Mac** *(inhibitory; responds to blue light)*
- **Arch** *(inhibitory; silences around 350 pA)*

It is likely best to being using ChR2 and NpHR, since they are known to work in *C. elegans*, and speed is not necessary for our application (the rise time for GCaMP3 is on the order of 100ms, and the decay time around 600ms). However, as with sensor molecules, this may change as the techniques develop, or new information is learned.

3.2 Optics

It is expected that the optogenetic stimulation will take place through the same microscopy setup as used for the activation readout (see section 1.1). However, to select individual neurons, instead of illuminating an entire animal uniformly, we may use a similar setup to the DMD system currently in the Samuel Lab. How this system will be integrated with the spinning-disk confocal microscope is, as far as I can tell, currently unknown. It may be possible to do even better than the DMD by taking advantage of the spinning disk itself. This is a question that should ideally be taken up by somebody who knows more about optics than I.

3.3 Genetics

If perfect 3D selectivity in stimulation is not possible optically—as it probably won't be—we may also use some genetic tools to limit the subset of cells expressing opsins.

3.3.1 Genetic Mosaic

One possibility, suggested by Ed Boyden, is the generation of genetic mosaics: this is also not a technique that I understand fully, but essentially, some transgene is introduced in such a way that at every cell division in the lineage, there is some probability that the transgene will not be copied, and will only continue down one branch of the lineage. If this procedure is performed sufficiently many times, and a sufficiently good screen is developed, then in theory, we can isolate worms expressing opsins only in one neuron at a time, and sort them out by which neurons are labeled (then we can use these worms as a basis to probe the effects of the labeled neurons on the rest of the nervous system).

3.3.2 Identification of Promoters

An alternative, more traditional approach, is to identify promoters that are specific to certain limited subsets of cells, in combination with the optical techniques above, to achieve functional single-cell resolution. (That is, some of the selection can be done spatially, and some could be done genetically.)

4 Verification

Goal: Determine the accuracy or fitness of a given model

"How will you know when you are done?" is a question I am commonly asked regarding this project. Below is my current thinking on this topic.

4.1 Quantifying Behavior

One possible approach is to make well-known behavioral assays (in chemotaxis, thermotaxis, etc.) testable algorithmically and quantitatively from vision tracking data. We could then reproduce these assays in a virtual environment with our modeled worms and see that the numbers fall well within the standard distribution of a population of real wild-type worms.

4.1.1 Biophysical Simulation

To do this, we must model not only the nervous system, but also, to a certain degree, both the body and the environment of *C. elegans*. Suzuki and Ohtake [**?**] have a simplified body model of *C. elegans*, as does Lockery [**?**]. One of these might form a good starting point.

4.2 Predictable Perturbations

Perhaps a more tractable approach than quantifying the wild-type behavior is to produce predictive results regarding defective animals.

4.2.1 Mutants

Several mutants, such as *unc-3*, *unc-6*, *unc-30*, *unc-76*, and *unc-86*, have well-characterized behavioral defects in addition to well-characterized defects in neural morphology (the presumptive cause). Ideally, given these defects in neural morphology, our model should be able to predict the behavioral defects.

4.2.2 Laser Ablation

In addition to the relatively small space of genetic mutants with well-known behavior and neural defects, we can also introduce arbitrary neural defects by killing neurons with laser ablation, and performing behavioral assays to see if the behavior of such animals matches a prediction by our models.

4.2.3 Laser Inhibition (Halorhodopsin)

If we can genetically and/or optically isolate a given neuron for optogenetic stimulation during behavior (see section 3), then it may be more desirable to transiently inhibit the activity of such a neuron than to kill it, for the purpose of generating more nuanced data to be matched against a model's prediction.