Program and Visualized *C. elegans* Neuron Network in Optogenetics

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Abstract

All the time, we are curious how brain learn new thing and then memory. Memory is basal for higher level brain process. Scientists design lots of behaviour experiments to find out how subject anmimal response to some thing and form memeory to recall the behaviour. Behaviour experiments on human, monkey etc, brought lots of knownledge for us. However, here is not enough clear research to show the neurons network dynamics during learning and form memory while neurons is the material base of memory. Here we use optogenetic tools to train *C. elegans* neuron AWA and surrounding neurons by light-sensitive ion channel CoChR, and image the neurons network activities by Ca^{2+} indicator GEM-GECO. We wonder what is the neurons network dynamics during train, and even decode this pattern.

Purpose

Brain is made of lots of neurons, which are connected by synapses. These neurons and synapses form our memory and consciousness. Many scientists work hard to try to understande it, so we get knowledge, such as, action poen-

tial, synapses, sensation and etc. One idea think that our memory is form by the different neurons network connected by synapse. So here are a great project provided the connectomes of *C. elegans*. Connectome show the physical connection between of all 302 neurons in the *C. elegans*.(ref.1) But connectome is a static picture, which is not enough to show the how neurons' activities sense environment and control the behavior . One possible method is to observe living neurons during learning.

Nowsday, scientists develop lots of fluorescent protein and light-activated ion channel. In other words, if these parts are used in neurons, they will be controlable input and output components. So we want to figure out the dynamics process during *C. elegans* is trained. We choose a group neuron called AWA and other neurons. The fluorscence protein GFP , GEM-GECO is transfered to wt *C. elegans*(ref.2,3). Here I wonder whether is different after tran. I it is difference, how it change during the process of training.

My hypothesis is that the neurons network activities of AWA will modified by synapse during training. In our experiment, we will see the fluorescence change of neurons between AWA and alcohol neurons.

To prove the neurons activity has changed, we do it step by step.

Firstly, we proved that transgene *C. elegans* function as wild type *C. elegans*. In other word, when we use light on the transgene *C. elegans*, which perform similary to the response with dictycle.

Secondly, we will test whether calium indicator GEM-GECO work. And observe whether GEM-GECO and CoChR work together.

Lastly, we start the train experiment. We will observe the population behavor and neurons acivities.

Based on the experiment results, we will get the conclusion. If If we succeful to oberse the temporal-spactional pattern, we may in get the idea how the neurons form memory and how the train modify the neuron network.

Background

Brian is complex structure, and has complex functions. One of the most intriguing of the brain's complex functions is the ability to store information provided by experience and to retrieve much of it, either consciously or unconsciously.Without this ability, many of the congnitive functinos caould not occur. *Learning* is the name given to the process by which new information is acquired by the nervous system and is observable through changes in behavior. *Memory* refers to the encoding, storage, and retrieval of learned information.(ref .1 textbook). Here is a well-known operant conditioning experiments called Skinner box. Skinner Box, invented by Burrhus Frederic Skinner, is a laboratory apparatus used to study animal behaviors. Inside this box, when the mouse(or other subject animal) peroform correct response to specific stimuli, such as light or sound signal, this box delivers food or other reward. Skinner box allow experimoters to perform stduies in tranning through reward. However, here is a black box between stimuli and behavior, and also the neurons network of mouse brain or human brain is too complex and difficult to perform experiment. So we want to perform skinner box-like experiment on *C. elegans* to study the neuron network dynamics pattern during trainning.

Caenorhabditis elegans, a classical model organism, is widely used in scientific researchs. *C. elegans* is a free living,transparent nematode, about 1mm in length. At 20°C, the laboratory strain of *C. elegans* has an average lifespan around 2-3 weeks and a generation time of 3 to 4 days. When Sydeney Brenner first time proposed using *C. elegans* as a model organism, he wanted to investigate neural development in animal promarily. For us, here are three key points that we choose *C. elegans* as the subject animal.

Firstly, *C. elegans* is one of two organism whose connnectome is found completed. Based on the physical connect between neurons, we can easy and reasonable to choose target neuron for study the neuron network. Secondly, *C. elelgans* only has 302 neurons in hermaphrodite. It is suitable and friendly to do genetic expeiment and analysis. Last point is transparent body of *C. elegans*, which mean that light can throught cell body to neuron and neuron out to cell body. Using fluorescent protien and lighat-activeted ion channel which are well developed recently, we can preciesly control neurons and quanlity measure the neurons activity.

One kind of fluorescent protein called GEM-GECO is used. GEM-GECO belong to the family of G-CaMP, whose fluorescence will change dependent on the concention of calcium. From 1990s, just after R.Y. Tisen mutasis GFP, Tisen and other scientists try to component GFP and other part to image calcium. Typically, G-CaMP consists of tandem fusions of a blue- or cyan-emitting mutant or the green fluorescent protein(GFP), calmodulin, the calmodulin-binding peptide M13, and an enhanced green- or yellow-emitting GFP. Binding of calcium ion makes calmodulin wrap around the M13

domain, increasing the fluorescence resonance energy transfer(FERT) between the flanking GFPs.(ref.2) Fluorescence of GEM-GECO will show the activity of neurons.

Another important part is the light senstive ion channel, while CoChR is used in our project. CoChR enable us to stimule neuron in millisecond-scale temporal precision. CoChR is blue-light sensitive light senstive ion channel. CoChR is chosen because it is five times more sentive to blue light than the commonly used ChR2.

(*Which lab do the same thing all over the world?*)(*How is the newest devlopment of this?*) (*What is largest challge problem in technology or others way?*)(*How other people in the past and now try to solve the problem?*)(*But how the memory for human and C. elegans is different. How use the C. elegans to representate human mind?*)(*What is the advatage of our try*)(*Why it must be import, why not intersting?*)

Research plan

1 Construction transgene *C. elegans*

str2 gene was chosen to expressed on AWA specifily. Fistly, we constructe the plasmids str2: CoChR:GEM-GECO:GFP. This plsmdis with minios system are transfer to wild type *C. elegans*(N2) by microinjection. After selecting, transgene worm str2::CoChR::GEM-GECO::GFP is stable heredity.

2 Test whether GEM-GECO and CoChR work

Before we train and image, function of GEM-GECO and CoChR should be checked.

To check GEM-GECO, confocal microscope observe the fluorescence of GEM_GECO. *C. elegans* are placed and limited on the dish. After 20s that confocal microscope capture the fluorscence(510nm), diacycle is added behind *C. elegans*.

Final results are representated by GEM-GECO fluorescence change over time. The fluorescence ratio is calclulate by ratio = ($\text{fluorescence}_{\text{current}}$ − $\text{fluorescence}_{\text{begin}}$)/$\text{fluorescence}_{\text{begin}}$. If GEM-GECO function well, we can find that the fluorescence will increase after adding diatycle.

Then transgene *C. elegans* will be test whether light stimuli can result in similar response as diactyle. Before stimuli *C. elelgans*, it stay inside a staright channel few larger than worm body in a customied PDMS microfludic chip. Head is moved to the center of camera. Then 470nm blue light(from fluorescence microscope) is truned on for 4s.

Final results is obtained from the movement of *C. elegans* during blue light turn on. If stimulied worm walk ahead or shake body rather than before stimulied, it mean that CoChR play same role with diactyle to active AWA neurons.

Last part, CoChR and GEM-GECO are checked whether work well together. Using confocal microscope, before stimuli *C. elegans*, living *C. elegans* is fixed inside a narrow channel inside microfludics. During capturing,

405nm laser turn on all the time to exite GEM-GECO, and microscope is set to receive 510nm emssion light of GEM-GECO. First 20s of experiment, 470nm blue light turn off; then 470nm blue light turn on for 2s to stimuli CoChR. Also, here are a few of control group with different light is used.

Data is processed as GEM-GECO test. If fluorescence of GEM-GECO only raise when *C. elegans* is stimulied with blue light. Or other calium indicator or light-senstive ion channel should be used.

3 Train *C. elegans* and Image the neurons

Here we take exmple of alcohol. Wild type *C. elegans* has not flavor on alcohol, we will train *C. elegans* with the flavor of alcohol, then image the neurons network during traning.

Transgene *C. elegans* train in a 6cm petri dish, which has NGM with 2.5μM ATR(all-trans-retinal). Then alcohol are added into dish, and dish were lighted by blue light for 2 hour. After 2 hour, we wash the plate to recover the worm in M9 buffer and put the mixture on one side of a new plate. Finally, we put the alcohol on the other side to see if the worms have the tropism of the alcohol. Control group is done by used N2 and different light.

Final behaviar results are representated by the ratio of worm moved to alcohol. If transgne *C. elegans* has higher ratio than control group, it will be reasonble to think that *C. elegans* get the flavor (memory) of alcohol.

Now we will open the black box of above train. The trainned *C. elegans* will be imaged the neurons network actvities.

References

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