Protocol
Experiment 2 Electrophysiological recording on earthworm

1. Background:

The earthworm is ideal for simple action potential (AP) recording. The linear anatomy enabling a direct and easy way to measure the axon length and the conduction velocity of action potential.

As shown in the figure below, the anatomy of earthworm is quite straightforward. The nerve cord is near the ventral surface of the worm. The giant axons appear as three large profiles (gf) near the dorsal surface of the cord, seen better in the close-up figure. The median giant axon in the center is larger (and conducts more rapidly) than the two lateral giants next to it. The remainder of the nerve cord is largely neuropile (np), where synaptic connections are made. For more information, please check the references and other materials online.

In this experiments, you will be able to perform simple experiments on earthworm action potential recording in different conditions and calculate the conduction velocity from your recordings. You will also practice basic signal processing process and coding techniques to dig out the useful information from your results.

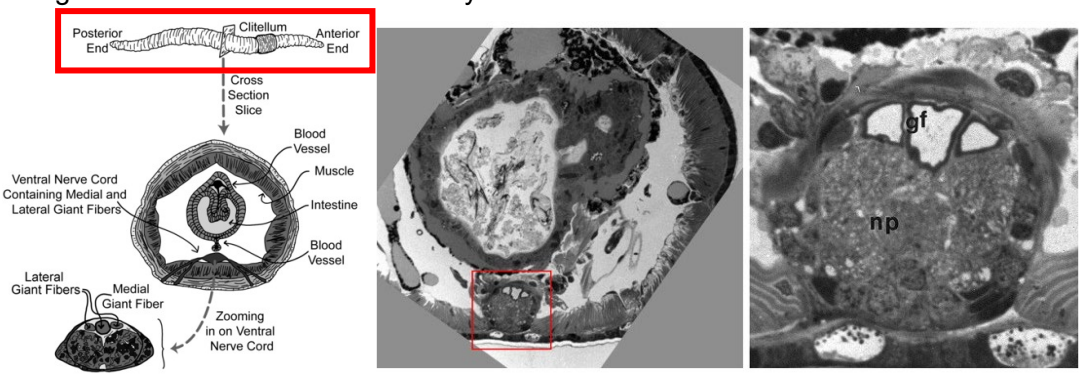

Fig 1. Anatomy of GF in earthworm

You should be able to differentiate the anterior end and posterior end by observing the position of clitellum (a thickened glandular and non-segmented section).

References:

https://www.science.smith.edu/departments/neurosci/courses/bio330/labs/L4giants.html
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4116350/

2. Experiment design:

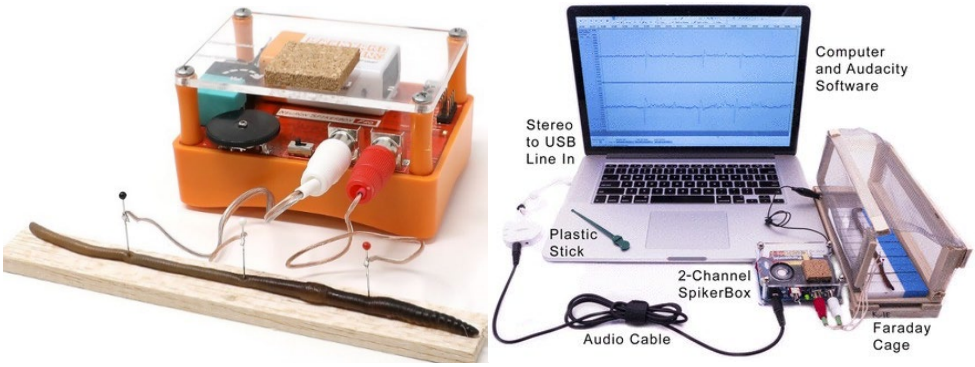

Fig 2. Experiment setup

Fig 2 shows the experiments setup. A two-channel Neuron Spikebox Pro (Backyard Brains, Inc.) is used to record the action potential. Please check the inner circuit of the spikebox in the following website (optional):
https://backyardbrains.com/products/files/Neuron.Spikerbox.Pro.V1.pdf

You are required to download the software BYB Spike Recorder before our experiment section. The signal will be recorded and saved as Waveform Audio File (.wav), and you will be required to convert the .wav file to a readable plot in Matlab (recommended).
Link to download BYB Spike Recorder:
https://backyardbrains.com/products/spikerecorder

2.1 Experiment materials

Please check you have all materials before starting the experiments.
Electronics: 1 Neuron Spikebox Pro, cable set (1 black USB cable, 1 green 3.5mm audit cable, 2 electrode white-2 pins, red-1 pin, 1 hook-to-hook clip, 3 crocodile-to-crocodile clips), 1 faraday cage
Consumables: 1 balsa wood, 1 plastic tweezer, 1 plastic probe, 1 plastic pipette tip, 1 pencil, 1 ruler, 3 beakers, 3 cotton buds
Chemicals: 10% ethanol alcohol, DI water, Iced water

2.2 Experiment 1

Compare conduction velocity in the anterior and posterior side of the giant axion under mechanical stimulation

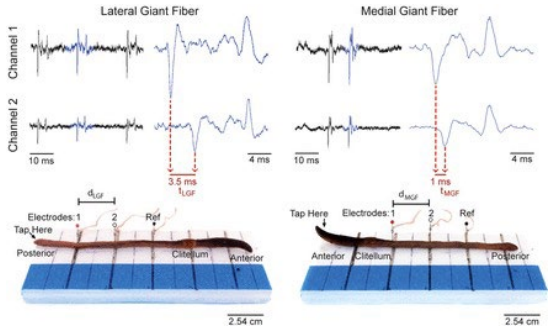

Fig 3. The action potential recorded from different side of the earthworm

i. The anesthesia of earthworm will be performed under 10% ethanol solution for 4min. The anesthetic effectiveness can be determined by a lack of movement and a cessation of the escape withdrawal reflex by tapping the head to the worm. After anesthesia, briefly rinsed the earthworm in water and then began the experiments.
ii. Place the earthworm on a piece of balsa wood, and insert the three electrodes into the worm slightly off its centerline so as to try to avoid piercing the intestine or ventral nerve cord. (E1 to E2: 1.5cm; E2 to E3 (G): 3cm)
iii. Place a faraday cage around the earthworm, and clip the caraday cage to the ground using a hook clip cable. Plug the electrodes into the Neuron Spikerbox Pro and the USB cable into the PC. Record real-time AP signal in BYB recorder software.
iv. Lightly tapped the very anterior end of the earthworm with a plastic probe. Record for 30s to 1min. Change the electrode position to posterior direction, repeat the tap and recording.
v. Check the time delay in a recording application software (eg. Audacity) or in your Matlab plots.

Link to download Audacity:
https://www.audacityteam.org/

2.3 Experiment 2
Dependency of conduction velocity – temperature
i. Soak the earthworm in the ice bath for 30s;
ii. As the worm cools down over time, repeated measurements of the conduction velocity can be performed;
iii. Calculate the conduction velocity and compare with your previous result.

References:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3597421/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4116350/

Useful videos: (Please watch the videos before experiment section)
https://www.youtube.com/watch?v=3_B7kCjs4jg
https://www.youtube.com/watch?v=qZFTuOxUVok
https://www.youtube.com/watch?v=RTGnzswzqm4&t=4s

Appendix

1. Data acquisition:

For experiment 1 (anterior/posterior)
Report the anesthetic time for earthworm, distance between electrodes, and the digital filter setting in the BYB software (if you changed the default setting).
Record signal from both anterior and posterior side

For experiment 2 (temperature)
Report the dipping time in ice water.
Record signal, compare with the result from experiment 1.

If you fail to complete any one of these experiments, please propose the reasons, and give your anticipation for the possible results (including conduction velocity and SNR change, as well as other aspect based on your own knowledge)

2. Lab Report

Data collection is shared within a group. Each member analyzes their data and submit a lab report **individually**. There is no page limit.

Background and experiment protocol is not required. Please follow the given structure for lab report:

Lab report outline:

I. Top of your report

- ✧ Module code – BN5209
- ✧ Experiment title - ENG Earthworm Nerve Conduction
- ✧ Student name and ID
- ✧ Group number
- ✧ Date

II. Results and discussion

- ✓ Table 1: Conduction velocity calculated from each experiment [4 pts]

(Room temperature: Anterior and Posterior; Ice bath: Anterior and Posterior)

- ✓ Plot the power spectral density for each electrode. [6 pts]
- ✓ Plot representative raw ENG signals from room temperature conditions (Anterior). Show both electrodes in the same plot. [4 pts]
- ✓ Describe the known physiological principles that predict the changes innerve conduction velocity based on temperature. Do your results match expectations? [3 pts]
- ✓ Answer the question: What is Faraday cage? Why do we need a faraday cage? Explain its function based on your data and plots. [3 pts]

3. Coding guideline

(You are suggested to use matlab for the coding)

Please directly search on the MathWorks website (or other resources). You will find the code and examples. Please verify the code and choose one that meet your requirement. Here are some examples:

1. Read the .wav file

e.g. https://www.mathworks.com/help/matlab/ref/audioread.html

2. Digital filter

e.g. designfilt funtion: https://www.mathworks.com/help/signal/ref/designfilt.html
butterworth filter design: https://www.mathworks.com/help/signal/ref/butter.html

3. Power spectral density

e.g. https://www.mathworks.com/help/signal/ug/power-spectral-density-estimates-using-fft.html

4. Cross correlogram

e.g. https://www.mathworks.com/help/matlab/ref/xcorr.html
https://www.mathworks.com/help/econ/crosscorr.html