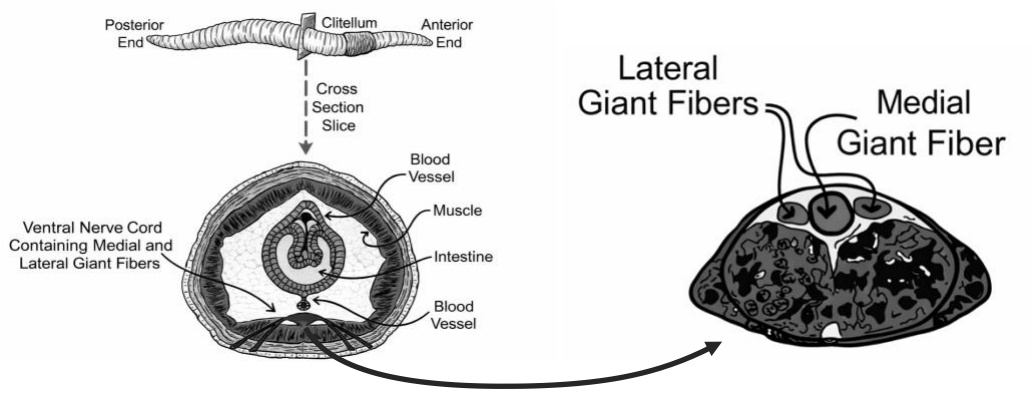
BN5209 – Lab: Earthworm Nerve Conduction

SEM2 AY2025/2026

Overview

The earthworm is a convenient model for basic action potential (AP) recordings and for examining how temperature influences nerve conduction. Because lower temperatures are known to slow neural signal propagation, the earthworm's simple, linear body enables direct measurement of axon length and straightforward calculation of AP conduction velocity across different thermal conditions.

As shown in the figure, the earthworm's anatomy is relatively simple. The ventral nerve cord lies close to the ventral surface, facilitating external access and controlled cooling. Within the cord, three giant fiber axons are visible, with the central (median) giant axon being larger and typically conducting faster than the two lateral giants. Because these axons are large and superficially located, changes in temperature produce clear, measurable slowing of conduction velocity, making the earthworm well suited for demonstrating the biophysical effects of cooling on neural signaling.



Adopted from: Shannon *et al*. Adv Physiol Educ. 2014. doi: 10.1152/advan.00088.2013.

In this lab, you will:

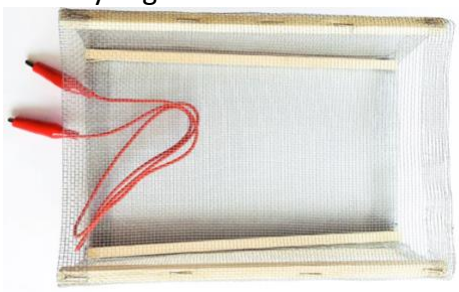
1. Setup a neural recording system to record nerve action potentials from an earthworm.
2. Calculate and compare conduction velocities at different thermal conditions
3. Perform basic signal processing process and coding techniques

Materials

Materials provided:

1. Earthworms
2. BackyardBrains Neuron SpikerBox Kit

   - 1 Neuron SpikerBox
   - 2 red needle electrodes
   - 1 white needle electrode
   - 1 crocodile-to-crocodile clip
3. 10% ethanol bath
4. Cold ice packs
5. Balsa wood
6. Plastic probe
7. Faraday cage



Software students must install before class:

1. **BYB Spike Recorder (BackYardBrains)**
   - Download and install from the BackYardBrains resource page: https://backyardbrains.com/products/spikerecorder

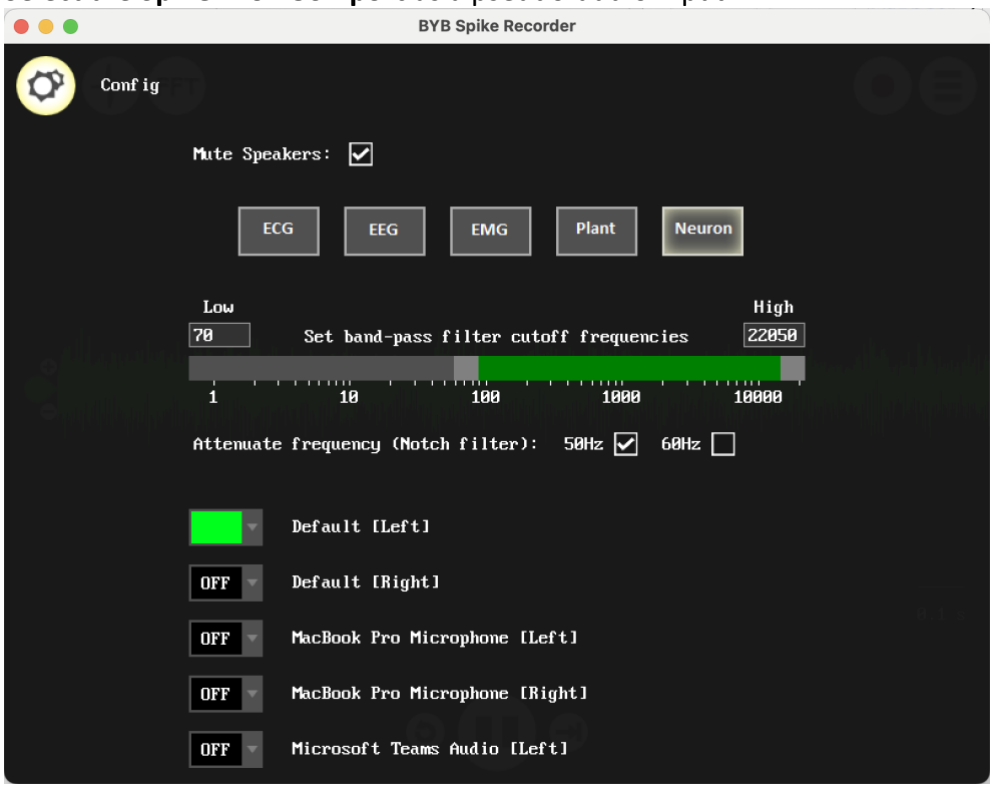
2. **Python (via Conda) or MATLAB**

Protocol

I. BackyardBrains setup

1. Plug in a BYB Neuron Spiker Box board to your laptop via USB

2. Run the BYB Spike Recorder software and go the Config menu to get the following settings:
   - Select **Neuron** to select physiologically relevant EMG frequencies
   - Select **50Hz** Notch filter to remove interference from Singapore's power grid
   - Select the **Spiker Box USB port** as a pseudo-audio input.



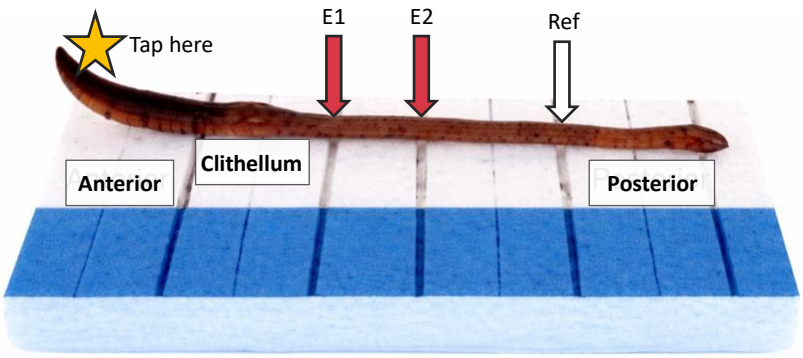
3. Use alligator clips to hook the faraday cage to ground.

II. Earthworm preparation 1: Thermal effects on nerve conduction

1. Submerge the earthworm in 10% ethanol for 4 minutes to anaesthetize
    - Confirm anesthetic state of the worm by checking absent response when tapping.

2. Place the earthworm on a slab of balsa wood as shown below

3. Insert three needle electrodes into the worm slightly off its midline to avoid piercing the intestine or ventral nerve cord.
    - E1 (red): 0.5 cm posterior to the clitellum
    - E2 (red): 1.5 cm posterior to E1
    - Ref (white): 3.0 cm posterior to E2



4. Record nerve action potentials under **ambient temperature conditions**
    - Click the **recording button** at the top right.
    - Lightly tap the anterior-most end of the earthworm with a plastic probe
    - Record for **10 seconds**, and end the recording by clicking the **recording button** again.

5. Record nerve action potentials under **gradual cooling conditions**
    - Place ice packs around the faraday cage
    - Record 10 seconds of data similar to step 4.
    - Keep collecting 10-second recordings at **1-minute intervals** until 10 minutes have passed.

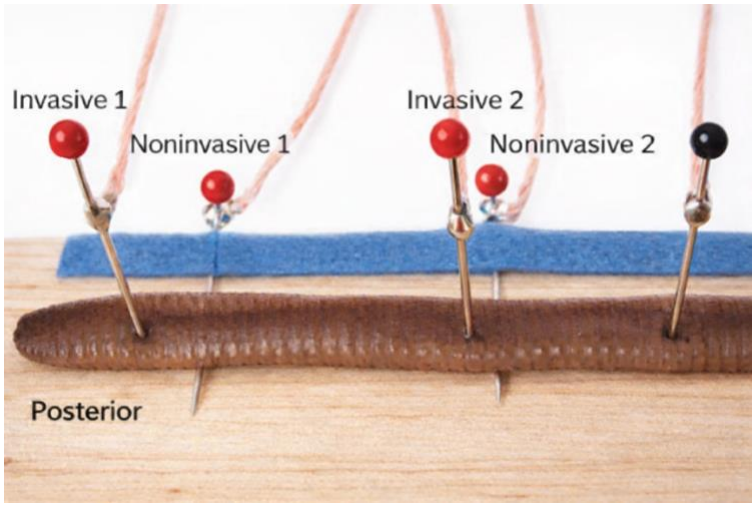
III. Earthworm preparation 1: Invasive versus Non-Invasive Recording

1. Obtain a new worm and anaesthetize in 10% ethanol for 4 minutes as previously described

2. Place the earthworm on a slab of balsa wood as shown below

3. Invasive electrodes: Insert three needle electrodes into the worm slightly off its midline to avoid piercing the intestine or ventral nerve cord.
    - Invasive 1 (red): 0.5 cm posterior to the posterior tip
    - Invasive 2 (red): 1.5 cm posterior to E1
    - Ref (white, common): 3.0 cm posterior to E2

4. Noninvasive electrodes: Place two needle electrodes under the worm.
    - Noninvasive 1 (red): 0.5 cm posterior to the posterior tip
    - Noninvasive 2 (red): 1.5 cm posterior to E1
    - Ref (white, common): Short to invasive reference needle



5. Record nerve action potentials under **ambient temperature conditions** for both invasive and non-invasive electrodes

Lab Report

Data collection is shared within a group. Each member is to analyze their data and prepare their lab report **individually**. There is no page limit. **Report due 2 weeks after lab session.**

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
- Temperature-dependence of Nerve Conduction
  - Plot the power spectral density for each electrode. **(1 pt)**
  - Plot representative raw ENG signals from room temperature conditions. Show both electrodes in the same plot. **(1 pt)**
  - Plot the temporal cross-correlation between the two electrodes at all measured cooling time points. Annotate the peaks to show the time delay τ between the proximal and distal electrodes. Show a cross-correlation plot at t=0, 1, 2, 5, and 10 min. **(4 pt)**
    - Y-axis: Cross-correlation
    - X-axis: Time delay
    - Title: Temporal cross-correlation at time = <*time_in_minutes*> min of cooling.
  - Plot the progression of nerve conduction velocity (NCV) throughout the cooling period, by dividing the inter-electrode spacing by tau. **(2 pt)**

$$NCV = \frac{\Delta x}{\tau}$$

    - Y-axis: NCV
    - X-axis: Cooling time, t (let x=0 be the initial room temperature condition)
    - Title: Cooling effect on nerve conduction velocity
  - Describe the known physiological principles that predict the changes in nerve conduction velocity based on temperature. Do your results match expectations? **(2 pts)**

- Invasive vs Non-invasive recording
  - Plot the power spectral density for each electrode. Have one plot each for invasive1/invasive2 **(1 pt)** and noninvasive1/noninvasive2 **(1 pt)** pairs of electrodes.
  - Identify the baseline signal (signal before mechanical stimulation) and measure the baseline RMS for the invasive **(1 pt)** and noninvasive **(1 pt)** pair of electrodes. Tabulate the number.
  - Identify the stimulation-induced signals and measure the signal RMS for RMS for the invasive **(1 pt)** and noninvasive **(1 pt)** pair of electrodes. Plot and compare the signal-to-noise ratio between invasive and non-invasive electrodes.

$$SNR = 20\log_{10}\left(\frac{RMS_{signal}}{RMS_{noise}}\right)$$

- Compare the SNR between invasive and noninvasive recordings. Do you results match expectations? Briefly explain in 1-2 sentences why such differences in SNR are expected between invasive and noninvasive recordings. **(1 pt)**
- Plot the temporal cross-correlation between invasive1/invasive2 **(1 pt)** and noninvasive1/noninvasive2 **(1 pt)** channels.
  - Y-axis: Cross-correlation
  - X-axis: Time delay, tau
  - Title: Temporal cross-correlation of <*Invasive*/*Noninvasive*> electrodes separated by 1 cm).
- Between invasive and noninvasive recordings, which measurement technique gave a clearer time delay peak? Do your results match expectations? Briefly explain in 1-2 sentences why such differences in the shape of cross-correlation between invasive and noninvasive recordings were obtained. **(1 pt)**

III. References

IV. AI Use Declaration