Lab 1: Principles of neuron electrophysiology measurements

0. Assignments associated with this lab

- Comprehension questions (section 4)
- You do not have to turn in your completed code. However, you are asked to provide the figures generated by running your analyses (last question of the comprehension questions).
    - All figures that must be provided are underlined in the lab manual and are flagged in the code templates
- **BIOE 566/EE 564 sections:** You must write an abstract that concisely summarizes the motivation, methods, results, and conclusions of your experiment (do not exceed 250 words)

1. Equipment required

- Hardware
    - Spikerbox pro
        - Connection cable to connect to computer
    - Computer with USB connection
    - Cockroach and equipment to prepare leg
        - Cup
        - Ice + water
        - Forceps
    - Electrodes wired to mono audio connectors (to connect to spikerbox)
        - 1 pair of small (size 000) pins
        - 1 pair of medium (size 00) pins
        - 1 pair of large (size 0) pins
- Software
    - Backyard Brains Spike Recorder
    - Matlab
    - Scripts outlines and functions for analysis

2. Background

This lab will give you hands-on experience with electrophysiology techniques for recording the activity of neurons in the central nervous system. We will perform experiments to probe how measurement setup influence the signals we acquire. These experiments will illustrate that voltage referencing (experiment 1) and electrode properties (experiment 2) both change the signals captured by electrophysiology measurements.

2.1 Referencing

Electrophysiology measures electrical activity in the body via a voltage measurement. Voltage reflects the difference in the electrical potential between two locations. A voltage is therefore measured by calculating the difference between signals measured on two electrodes: a *recording electrode* and a *reference electrode*.

How we select the reference for electrophysiology measurements will influence what biological signals are captured. This becomes clear if we consider two extremes:

1. Positioning our recording and reference electrodes *very* close to each other so that they pick up activity of the same neurons. The signal measured by the recording electrode S_{rec} and the signal measured by the reference electrode S_{ref} are approximately equal. Then the measured voltage, $V = S_{rec} - S_{ref} \sim= 0$.

2. Positioning our recording electrode in the brain, and our reference signal somewhere outside the body. In this case, S_{rec} and S_{ref} will certainly not be the same. But we will now have a different challenge. We will now measure differences in charge coming from *all* possible sources in the body. Electrical currents produced by a single neuron are quite small (producing voltages in the microvolt to millivolt range). These small signals cannot be detected well among all other sources of electrical fields in the body when the reference voltage is too far.

In both configurations, we would see signals suggesting there are no neurons present. This is not true. It's simply that our reference set-up does not capture them.

In this lab, we will perform an experiment to systematically vary the placement of recording and reference electrodes and quantify how it influences the properties of our resulting measurements.

2.2 Electrode properties (Size)

Electrophysiology measures the voltage via an electrode. An electrode is a conductive sensor. The properties of this sensor—material it is made of, size, and so on—will influence how charge flows into the sensor, and thus influence our measured voltage. The detailed physics of how electrode properties influence measured signals is beyond the scope of this course. There is still active empirical and theoretical research (e.g. [1]) in this area. However, we will explore how one property—electrode size—influences our measurements.

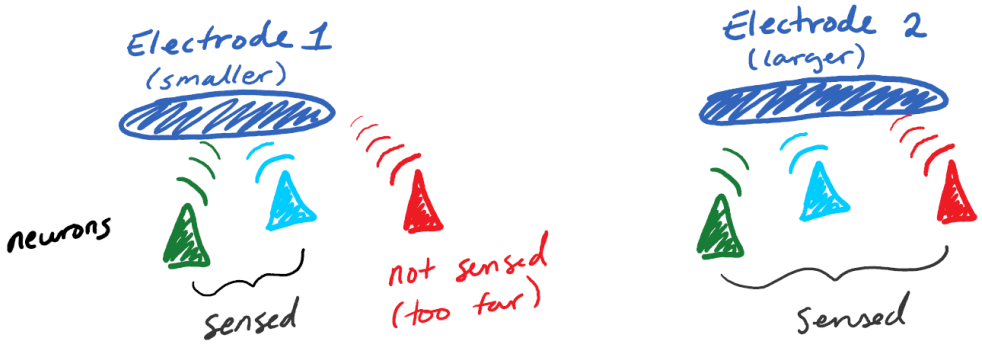

Fig. 1 Cartoon illustration of why electrode size influences electrophysiology signals.

We can get an intuition for why electrode size matters by considering the physics of electrical fields (and therefore current flow) and geometry of our measurements. Recall that the electrical field of a point charge Q falls off quadratically with distance from the source (r): $E \propto \frac{Q}{r^2}$ This rapid fall-off is a large part of why electrophysiology can isolate activity of neurons. Neurons far from our electrode generate such small electric fields that they are negligible compared to those of close neurons. However, let's now consider the geometry of our recordings as we change the electrode's size. As the cartoon in figure 1 illustrates, the number of neurons that are "close" to the electrode is a function of its size. The number of neurons whose action potentials contribute to the measured voltage increases as electrode size increases.

It is important to note that an electrode's size changes several other properties that will influence our measurements. The impedance (and resistance) of an electrode will decrease as it becomes larger (because larger electrodes have more surface area to collect electrons). Impedance will influence the current flow through the electrode and its noise properties. Lowering the impedance, in general, reduces both the loss of

signal due to shunting (current that flows to ground rather than through your amplifiers) and the thermal noise of your measurements [1-3]. Because of this, the *signal-to-noise ratio (SNR)* of an electrode is generally better with lower impedances. There is thus a trade-off between the spatial specificity of our measurements (achieved via smaller, higher impedance electrodes) and the measurement SNR.

In this lab, we will perform measurements to systematically vary the electrode size and quantify how this influences the resulting measured signals.

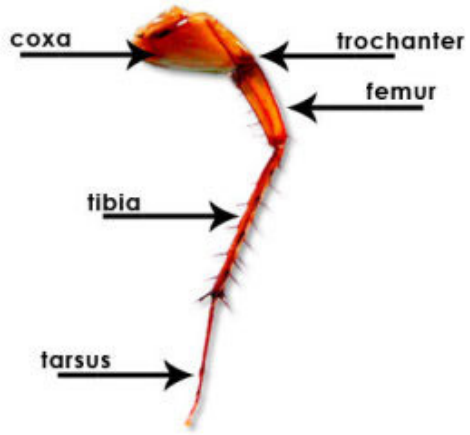

Fig. 2 Cockroach leg anatomy. Taken from https://animals.howstuffworks.com/insects/cockroach1.htm

2.3 Cockroach physiology primer

We will perform recordings on cockroaches, which have convenient physiology for exploring basic electrophysiology. We will record activity from the nerves in the cockroach leg. The anatomy of the leg is shown in figure 2.

The nerve and its sensorimotor processing can function separate from the body for a period of time (several hours). We will remove the leg for our experiments. Don't worry, the leg is designed to break easily and can regenerate as part of the cockroach's survival mechanisms. We will anesthetize the cockroaches before the removal procedure by cooling its body.

2.4 SpikerBox data acquisition system

We will use the SpikerBox Pro (from Backyard Brains) to acquire our electrophysiology signals. The SpikerBox performs amplification, which is critical for being able to detect the very small voltages associated with neuron measurements. It can measure up to 2 electrode inputs at a time, sampled at 1 kHz. The signals are read into a computer via USB input. The Spike Recorder software (from Backyard Brains) can read the signals and save it to disc. A schematic of the SpikerBox is available at each of your lab bench stations.

2.5 Electrophysiology signals and analysis

Our electrophysiology measurements will detect action potentials from neurons. Action potentials are very rapid, and therefore appear as high-frequency oscillations in our signal. If we have done a good job of isolating our *signal* (the action potentials) from the *noise* (everything else), they will also have a larger amplitude than the background signal. An example is shown in Fig. 3.

If we zoom in on one of those action potentials (*spikes*), they have a clear shape, an example of which is shown in Fig. 4. This is referred to as the *spike waveform*. Recorded spike waveforms may not look the same every time. This happens for several reasons. One is that background noise produces random distortions in the spike shapes; another is that spikes are sometimes emitted as groups or bursts; and a third is that you are probably recording from more than one neuron at once, and there are differences in the shapes of the waveforms that the electrode picks up. If the properties of your electrophysiology preparation (referencing, electrode properties, etc.) provide spatially localized signals, it can be possible to *isolate* spikes that occur from different neurons recorded on the same electrode. This is done by separating spikes by their waveform shapes. This is commonly called *spike sorting*.

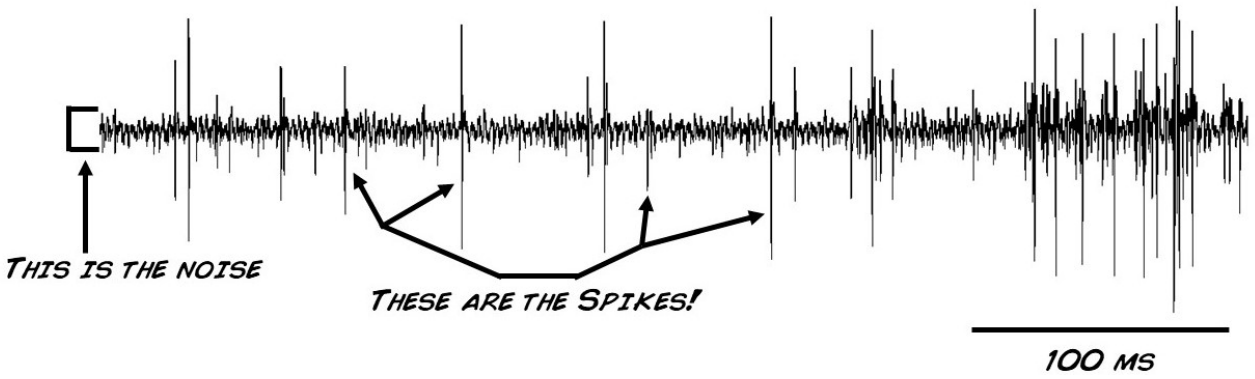

Fig. 3. Example electrophysiology trace from a cockroach leg nerve. Taken from https://backyardbrains.com/experiments/spikerbox#prettyPhoto

Spikes are typically detected using *thresholding*. Because they are larger in amplitude than the background signal, spikes can be found when the signal crosses a set amplitude threshold. The most common way to set this threshold is based on the statistical properties of the signal itself. If spikes are rare events (which they usually are), we can calculate the mean and variance of the time-series, and set the threshold to find these rare events (far from the mean).

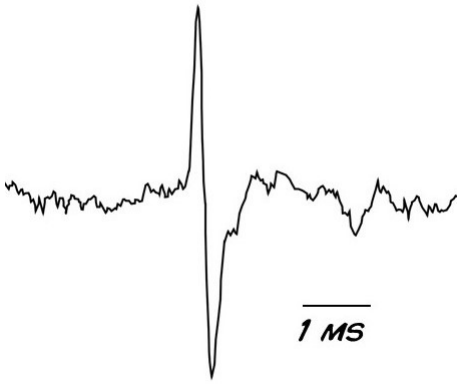

Fig. 4. Example action potential ("Spike") waveform. Taken from https://backyardbrains.com/experiments/spikerbox#prettyPhoto

3. Experiments

IMPORTANT NOTE: Read all provided procedures and safety information in Appendix S before starting experiments. You must submit the safety quiz for this lab via Canvas prior to beginning any procedures.

3.1 Experiment setup

SpikerBox setup

- Plug the SpikerBox into the computer, plug in the battery, and turn it on (via the 'Volume' knob)
  - Keep the volume knob low, please!
  - You should see a green light indicating the hardware is on
- Plug in the large electrodes to channel 1
- Open the Spike Recorder software and confirm you are streaming correctly
  - A USB icon should appear on the software if it is properly detecting the SpikerBox
  - Try wiggling or touching the electrodes (don't poke yourself—they're sharp!) to test if you see changes on the signal stream in the software
    - Note: Does the fact that moving the cables generates signal tell you anything about possible sources of noise in your measurements?

- Try taking a test recording and confirm that it saves a file

Fig. 5 Cockroach cooling, taken from https://backyardbrains.com/experiments/spikerbox#prettyPhoto

prepare the cockroach leg

- Now, we're ready to prepare the cockroach leg.
- Select a cockroach (in a closed container)
- Get a cup of ice water, some paper towels, and your forceps. Feel free to wear gloves if you prefer.
- Submerge your cockroach in the ice water as shown in figure 5. As the cockroach is cooled, it will be anesthetized. Wait ~1 minute, or until the cockroach stops moving completely.
    - Tip: Many people find it easiest to add the ice/water to the container with the cockroach. Make sure your cockroach is near the bottom of the container and then slightly open the top and pour ice + water in.
    - Be sure to have all tools ready before anesthetizing the cockroach, as you will need to act quickly before it wakes up.
- Remove the cockroach from the ice water and place on your paper towels.
- Remove one of the back legs at the level of the femur as shown in Fig. 6
    - Use the forceps to hold the coxa of the leg securely
    - Gently tug on the leg to break it off. This is typically most easily done with your fingers. Take care not to damage the leg with excessive force.
- **IMPORTANT:** Once you've removed the leg, place your cockroach back into the container and secure the lid. Once it warms up, it will wake up and make a nice recovery.
- Use one of the provided pins to secure your cockroach leg to the cork board on the SpikerBox. Place this pin at the far proximal end of the femur. This makes It easier to place recording electrodes.

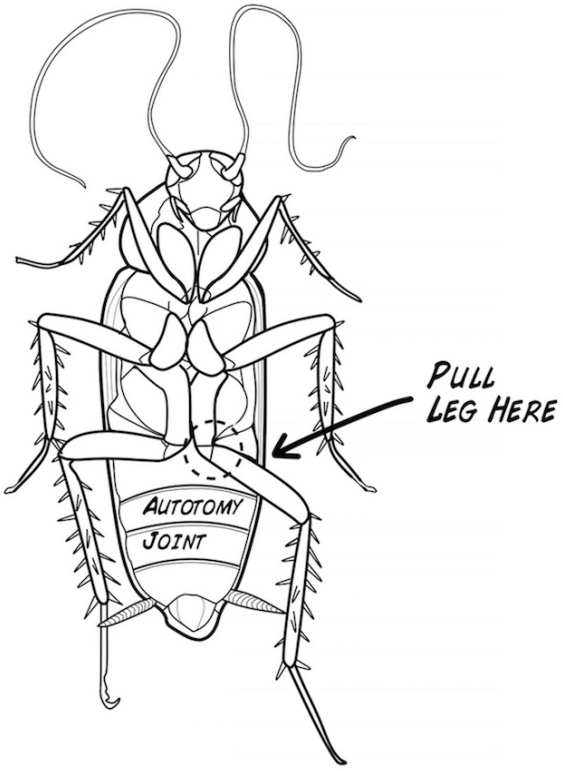

Fig. 6 Cockroach surgery illustration, taken from https://backyardbrains.com/experiments/spikerbox#prettyPhoto

Take a test recording

- Using the large electrodes you already plugged in to the Spikerbox, place the reference electrode in the femur (far end, close to where you put your anchor pin), and your recording electrode into the tibia.
- Use the oscilloscope on your screen to examine your recordings
    - You can adjust the vertical scale using the +/- buttons on the side
    - You can adjust the horizontal (time) scale with the scroll wheel
- You can also turn up the speakers a bit to listen to your signal

    - Tip: spiking activity, because it is very high-frequency, sound like sharp pops.
- When you believe you're successfully recording neurons, ask Professor Orsborn or the TA to come check your set-up.
- One common issue with electrophysiology is *line noise*—picking up 60 Hz oscillations in voltage that come from the AC power used in our electronics. This would look like big oscillations on your signal and sound like loud buzzing. This is usually due to poor grounding on your recordings. If you have this problem, consult with your TA or Professor Orsborn to debug.

Load a test file

- The final step to confirm our successful set-up is recording a test file and loading the data to confirm the file is accurate.
- Use the Spike Recorder to take a short (~15 seconds) recording of neural activity.
- Open Matlab and navigate to the directory where your file was saved
- Now load the data from the file into Matlab
    - Hint: Spike Recorder saves a '.wav' audio file. Look at the help for the Matlab function `audioread`
- Plot 2 seconds of your data. Inspect to assure you're convinced you're able to save files and load them correctly.

We're now ready to begin our experiments. Because the cockroach leg remains viable for a finite amount of time, we strongly recommend that you make the measurements for experiments 1 and 2 described below before shifting focus to analyzing the data in Matlab.

3.2 Experiment 1 – impact of referencing

We will conduct an experiment to explore how referencing influences the signals we measure. We will test the ***hypothesis*** that the spacing between the reference and recording electrode influences the number of neurons recorded and the spatial specificity of our recordings.

Data collection

- Take recordings of neural activity as you vary the position of your reference electrode (leave the recording electrode in the same place). Place the recording electrode into the tibia. Take recordings with at least 4 reference locations:
    - At the proximal (far) end of the femur
    - At the distal end (near the tibia) end of the femur
    - In the tibia, far from your recording electrode
    - In the tibia, close to your recording electrode
- Also explore other referencing positions. What if you tried the "extremes" outlined in section 2.1?
    - E.g. place the reference electrode in the corkboard
- Use the table provided at the end of this manual to keep track of your files and associated metadata. You will need this for your analysis.
- Listen and watch your signals for each recording. What changes do you notice? Note down observations in your table.

Data analysis

- We have provided an outline data analysis script called 'bioen466_lab1_winter2025_analysisExp1.mat'. Use this script to work through your data analysis

- The key steps of your analysis will be:
    - Load a file
    - Detect action potentials within your data
    - Calculate the rate of spikes
    - Assess the waveforms of your action potentials (amplitude, variance)
- The key plots you will generate through this script include
    - Firing rate as a function of electrode spacing
    - Average waveform as a function of electrode spacing
    - Amplitude of the waveform as a function of electrode spacing
    - Distributions of waveform amplitude for each recording
- We have also provided example plots to help guide your analysis.

3.3 Experiment 2 – impact of electrode size

We will conduct an experiment to explore how electrode size influences the signals we measure. We will test the ***hypothesis*** that the electrode size influences the signal to noise ratio and the spatial specificity of our recordings.

Data collection

- Take recordings of neural activity as you vary the electrode size (while keeping the reference and recording electrode spacing consistent). For this test, we will use a reference electrode placed in the femur and the recording electrode in the tibia. Make recordings using the 3 provided electrodes:
    - Large (Size 0)
    - Medium (Size 00)
    - Small (Size 000)
- You may also want to explore other referencing positions with the smaller electrodes. Are there any key differences you notice?
- Use the table provided at the end of this manual to keep track of your files and associated *metadata*. You will need this for your analysis. Metadata is additional information about what was performed in an experiment that is not contained in the files themselves, such as which electrode size was used in each recording.

Data analysis

- We have provided an outline data analysis script called 'bioen466_lab1_winter2025_analysisExp2.mat'. Use this script to work through your data analysis
- The key steps of your analysis will be:
    - Load a file
    - Detect action potentials within your data
    - Calculate the rate of spikes
    - Assess the waveforms of your action potentials (amplitude, variance)
    - Calculate the *SNR* of your measured spiking activity
- The key plots you will generate through this script include
    - Firing rate as a function of electrode size
    - Example waveform traces and average waveform as a function of electrode size
    - Amplitude of the waveform as a function of electrode size
        - Distribution of waveform amplitude for each electrode size
    - SNR as a function of electrode size

4. Comprehension Questions

1. What physical quantity are we measuring in our experiments? How many sensors (e.g., electrodes) are required?

2. What would happen if you reversed the 'ground' and 'recording' electrodes? (1 sentence)

3. When one electrode is in the femur and one in the tibia, there is a lot of "background activity". The amount of background activity (increases, decreases) when the electrodes are close together (both in the tibia). Explain briefly (1-2 sentences) why.

4. In experiment 2, we found that the properties of recorded signals changed significantly with the properties of the electrodes. Summarize how the amplitude and signal-to-noise ratio (SNR) is impacted by electrode properties (bulleted list of findings). Are these findings consistent with the proposed hypothesis of the experiment?

5. Looking at the waveforms and distributions of peak-to-peak amplitude for your various recordings, we see that sometimes we see multiple categories of waveform (distinct peaks, different waveforms). What does this reflect? Is there a trend in what types of recordings produce these distinct peaks?

6. What is the largest source of error in your results? How might you **change your experimental design** to improve your results?

7. Based on what you learned about the impacts of electrode placement and size in lecture, describe the best measurement set-up to record highly localized neural signals (electrode placement, electrode properties). Does this agree or disagree with what you observed in your experiments?

8. Append all figures generated by running the data analysis scripts (underlined in the experiment description) to your comprehension questions.

5. References

[1] Viswam, V., Obien, M., Franke, F., Frey, U., & Hierlemann, A. (2019). Optimal Electrode Size for Multi-Scale Extracellular-Potential Recording From Neuronal Assemblies. Frontiers in neuroscience, 13, 385. doi:10.3389/fnins.2019.00385

[2] Neto JP, Baião P, Lopes G, Frazão J, Nogueira J, Fortunato E, Barquinha P and Kampff AR (2018) Does Impedance Matter When Recording Spikes With Polytrodes?. *Front. Neurosci.* 12:715. doi: 10.3389/fnins.2018.00715

[3] Robinson, D. A. (1968). The electrical properties of metal microelectrodes. Proceedings of the IEEE, 56(6), 1065–1071. https://doi.org/10.1109/PROC.1968.6458

Appendix A: Data logging table

Recording #	Electrode Size	Electrode positioning	Notes and observations

Appendix S: Lab Safety Considerations for Lab 1

This appendix describes the safety considerations for the procedures to be performed in this laboratory session. Read this appendix in full ***prior to beginning your experiments****. If you have any questions regarding the lab procedures or safety considerations, ask the TA or Professor Orsborn prior to starting your experiments.*

Once you understand these considerations in full, complete the safety quiz on Canvas to indicate that you have read it and understand the procedures. The quiz must be submitted prior to beginning your experiments.

Cockroach handling

- Cockroaches can harbor bacteria and other diseases. Our cockroaches—purchased specifically for laboratory experiments—present minimal risk, but should be handled to reduce risk of bacterial spread
    - Wearing gloves while handling the cockroaches is recommended
    - Wash hands thoroughly after handling the cockroaches and/or cockroach legs
    - Wash any tools used during cockroach leg removal thoroughly with warm water and soap
- Dispose of cockroach legs in the trash after completion of experiments. No cockroaches or cockroach parts are to leave N133

Sharps handling (electrodes and inspect pins)

The electrodes and inspect pins used for our recording and stimulation experiments are sharp needles. Follow proper protocols for handling and disposing of sharps for all electrodes.

- Always grab the pin from the capped (rounded) end
- Never grab pins close to or on the sharp end
- When not in use, store electrodes and pins with sharp tips placed into the provided protective foam
- When placing the electrodes into the foam, set the foam down on a hard surface. Do NOT hold the foam while returning electrodes, as this risks self-injury
- Electrodes or pins that are damaged or need to be disposed of must be thrown into a sharps container