Lab 2: Using electrophysiology to study neural systems

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 flagged in the code templates
- **BIOE 566/EE 564 sections:** You must write a methods section describing the techniques and equipment used in your experiment. You should use the IEEE conference paper template, and your methods section should not exceed one page.

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 (with connection to spikerbox)
  - Manipulator
  - Small probes for tactile stimulation (e.g. toothpicks)
- Software
  - Backyard Brains Spike Recorder
  - Matlab
  - Scripts outlines and functions for analysis

2. Background

This lab will give you hands-on experience with using electrophysiology techniques to interrogate the relationship between neural activity and sensorimotor inputs and outputs. We will perform experiments to probe how neural activity *encodes* tactile stimulation (experiments 1), explore properties of this encoding (experiment 2), and 3) how electrical stimulation of neurons evokes behavior (experiment 3). In experiment 3, we will continue to interrogate how the properties of our physical interface with the nervous system—properties of our electrophysiology set-up for stimulation—influence our results.

2.1 Input-output relationships in the nervous system

The nervous system operates by taking in natural stimuli (sound, vision, touch, etc.) and responding in the form of electrical action potentials among groups of neurons that ultimately change/guide the organism's behavioral output. For instance, sensory neurons in the sensorimotor system respond to the tactile stimulation of contacting an object. This tactile input is *encoded* in the activity of sensory neurons. The sensory neurons transmit this information to motor neurons, producing altered movements in response to the sensory stimulus.

Much of neuroscience and neural engineering focuses on understanding the input-output relationships of the nervous system. Understanding how sensory neurons encode touch may be critical for restoring tactile sensation in a prosthetic device. Building models of how motor neurons encode movements allows us to build "decoders" that can predict someone's intended movements from neural activity for a motor prostheses.

2.2 System identification

There are two common ways to explore the encoding of the nervous system. The first is to record the activity of neurons while presenting a variety of stimuli to understand the stimulus – neural response relationship. The second is to activate (or deactivate) neurons and observe the resulting behavioral response/output of the system. Each provides important clues about the overall input-output behavior of the nervous system.

These approaches are equivalent to performing *system identification* to understand the input-output relationships in the nervous system. One of the biggest challenges for neuroscience is that the input-output spaces for the nervous system are vast. Consider, for instance, all the possible types of tactile stimuli you could experience. Something could touch many different parts of your hand, the tap could be hard or soft, and so on. Sweeping all possible stimulus parameters is quite challenging. In your experiments, you will see that there are a wide range of neural responses and that they can vary in time.

System identification could be much simpler if the nervous system's response was linear. Recall that a system is linear if: $F(\alpha X + \beta Y) = \alpha F(X) + \beta F(Y)$. If a system is linear, the space of stimulus inputs we would need to sweep are much smaller, because more complex stimuli would simply be the combined response of simpler stimuli. In our experiments, we will test whether the nervous system responds linearly to stimulation inputs.

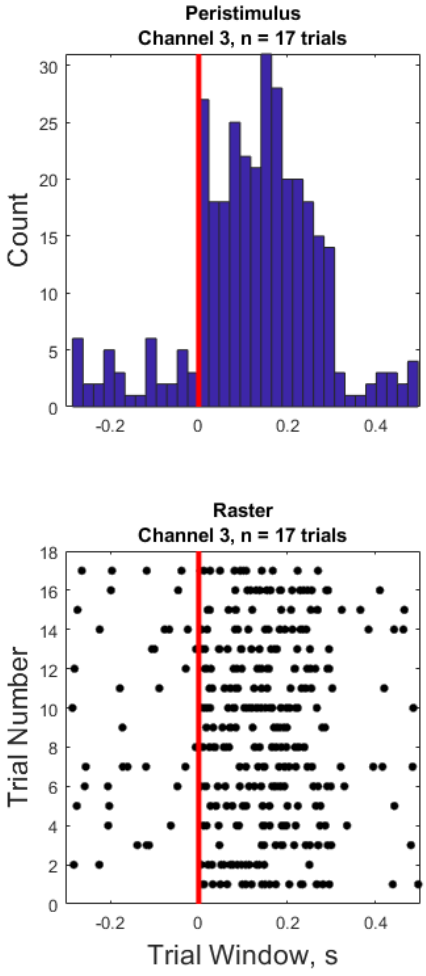

Fig. 1 Example raster plot (bottom) and PSTH (top). A raster shows the timing of discrete events (spikes) aligned to stimulus onset. A PSTH estimates the average spike rate aligned to stimulus onset. Taken from [1].

2.2 Rate coding and rate estimation

Neurons encode information in a variety of ways. One of the best-understood and well-characterized means of information encoding is called *rate coding*. Rate coding refers to encoding of information through the rate of action potentials. Most of our understanding of nervous system encoding is based around models that relate the firing rate of a neuron to stimuli and/or behavioral output.

Importantly, a neuron's firing rate must be estimated from our measurements of spike times. This is often done through a process called *binning*, where you define a time interval (a *bin*) and count the number of spikes in that interval to estimate a rate. We will write code to perform this operation to analyze our data in this lab. In future labs, we will revisit the importance of this estimation procedure for encoding (and decoding).

2.3 Raster plots and Peristimulus time histograms (PSTH)

A common way to visualize this response is called a *raster*, an example of which is shown in figure 1. A raster plots a tick-mark at

every time an event—in our case, an action potential—occurs. A raster thus allows us to visualize how spiking activity relates to external stimuli. Plotting this relationship across repeated presentations of a stimulus (as shown in figure 1) allows us to assess the consistency of the stimulus-response relationship.

The most common way to quantify a neuron's stimulus response is through a *peristimulus time histogram (PSTH)*. A PSTH estimates the rate of neural firing aligned to the time at which the stimulus was presented, averaged across all presentations of a stimulus. A PSTH is thus computed by 1) aligning spike times to the time of the stimulus for each trial (as is done for making a raster plot), 2) dividing the time axis into discrete time intervals (bins), and 3) calculating the average number of spike rate (number of spikes / unit time) in each bin.

As is shown in the example raster and PSTH, neurons typically fire action potentials even when no stimulus is presented. This is referred to as a *baseline firing rate*. The change in firing rate associated with the stimulus is therefore quantified by looking at change after stimulus presentation relative to baseline. As we will see in our experiments today and in future labs, the stimulus response may have temporal dynamics.

2.4 Electrical stimulation

Another way to probe neural function is through stimulating neurons and observing the resulting behavioral output. Electrical stimulation activates neurons by delivering current into the tissue. The current depolarizes neurons, causing them to fire action potentials. The detailed physics of how current injection evokes neural activity is beyond the scope of this course and an active area of experimental and theoretical research (e.g. [2]).

For the purposes of this lab, the key things to note are:

1) Current passed through the tissue depends on our electrode properties. Current flows into the tissue through one electrode (the *anode*) and flows out of the tissue through the other electrode (the *cathode*). The path of current through the tissue will therefore depend on the positioning of our electrode pair (recording and reference electrodes). Much like our recording of voltage, neural activation via electrical stimulation depends on the relative spacing of our electrodes.
2) Whether a neuron is activated by electrical stimulation of a given intensity (amplitude) depends on its size. Larger axons present more cross-sectional area to the current, so they are stimulated at lower stimulus intensities than are smaller axons. Thus, as stimulation current increases, the number of neurons recruited increases to neurons with a wider range of axon diameters.

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

Set-up our recording equipment and experimental subject:

- Plug the SpikerBox into the computer, plug in the battery, and turn it on (via the 'Volume' knob)
- Plug in your electrodes to channel 1. Select the size you prefer based on your data from lab 1.
- Open the Spike Recorder software and confirm you are streaming correctly and can save a file

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.

Take a test recording

- Place your recording and reference electrodes into the leg. Select positions based on your experience in lab 1.
- Use the oscilloscope and speakers to inspect your signals
- When you believe you're successfully recording neurons, ask Professor Orsborn or the TA to come check your set-up.
- Take a test recording and verify that it loads correctly in Matlab

3.3 Experiment 1 – Exploring stimulation-evoked responses

We will first explore using stimulation to probe neural system function. We will also explore how referencing influences our results.

Setup

- We will use the large electrodes to deliver stimulation
- Place your reference and recording electrodes both into the femur
- Connect your electrode to the spikerbox and confirm that you have good electrical contact (i.e. you are recording neurons)
    - We recommend positioning your leg so that it dangles slightly off the cork board
- Clip the stimulation cable to your electrodes and plug the audio jack into your computer
    - Turn down the volume on your spikerbox before doing this!
    - Once you clip the stimulation cable onto your electrodes, you might notice that you can no longer be able to see/hear your neurons anymore. This is expected—the stimulation cables interfere with the neural measurements. So, you will not record neural data while you stimulate.
- Test whether you can evoke movements by playing a song through your speakers
    - We recommend picking something with a decent amount of bass

    - We also recommend starting with the computer volume turned up to 100%
- You will know you succeeded when you see the cockroach leg move in response to the music

Probing frequency and amplitude-dependence

- We will now look at the evoked response in a more systematic way using a function generator to stimulate the leg at different frequencies.
- Open the NCH Tone Generator software on your computer
    - The interface allows you to play different waveforms through your computer's speakers.
    - Set the pulse type to a square wave
    - Use the dial on the bottom to change the frequency
    - Use the volume slider on the program to adjust the amplitude
- Vary the amplitude and frequency of stimulation and observe the resulting movements
    - Probe frequencies spanning at least 10 Hz – 5 kHz
    - Use the table provided in the appendix to note your results
    - In addition to marking move or not move, make note of the type of movement evoked (e.g. flexion versus extension of the leg)
- Test whether movement responses are linear in frequency
    - Pick to frequencies that evoke notably different movements
    - Use the signal generator to create an input that is a combination of both frequencies using the "add tone" button
    - Is the resulting response predictable based on your individual frequency responses? What does this say about the linearity of the response?

3.2 Experiment 2 – quantifying sensory responses to tactile stimulation

We will conduct an experiment to explore how neurons in the cockroach nerve encode tactile stimulation. We will test the hypotheses that the rate of neuron firing encodes tactile stimulation.

Data collection

Setup

- Position your recording electrode, placing the reference and recording electrodes into the femur, or choose your referencing configurations based on your results from experiment 1.
- (*Optional)* To increase the amount of data we can collect, if you want to, you can record from two sets of electrodes at the same time using channel 1 and channel 2 on the spikerbox.
    - Position your reference and recording electrode and connect to channel 1
    - Connect the provided second electrode to channel 2
        - position the recording electrode into a second recording site
        - Note: there is no reference electrode for channel 2 because our spikerbox uses one ground for both channels.
    - Use the settings panel on the SpikeRecorder to turn on channel 2
    - Confirm that you have neurons on both channels
        - You can listen to channel 1 or 2 with your speakers by toggling the switch near the speakers
- Take a recording to estimate the baseline firing of your neuron(s)

Identify tactile stimulation sites

- Identify at least two spines on the cockroach leg that evoke responses from the neuron(s) you are recording from
- Try touching different spines on the cockroach leg and listen to/watch your neurons to see if you can hear/see an obvious response.
    - Use the provided toothpick to precisely touch the spine
    - Use brief taps to the spine

Record tactile response

- We will now take recordings as we stimulate the leg
- In order to analyze our responses in relation to our stimulus, we need to note the time at which we present the stimulus. This will be done using the 'event logging' in SpikeRecorder. When you press a number on the keyboard, the SpikeRecorder will create an *Event* and record the time of the event (relative to the file).
    - Events will also be shown on the oscilloscope
    - When recording, events will be saved in a separate '.txt' file with the same name as your .wav file + "-Events" appended to the end.
- Another lab partner can help by running SpikeRecorder to log the events. Work out a system to log each time the leg is touched
    - We recommend practicing this a bit before collecting data
- Now record data to estimate your neuron's response to the following stimuli
    - Touching spine 1 (brief taps, allow 1-2 seconds between taps)
    - Touching spine 2 (brief taps, allow 1-2 seconds between taps)
- An important decision to make in collecting this data is how many stimulus presentations to use for your estimates
    - We suggest making several (at least 20) so that you can assess the trial-to-trial variability

Data analysis

- We have provided an outline data analysis script called 'bioen466_lab2_winter2025_analysisExp2.mat'. Use this script to work through your data analysis
- The key steps of your analysis will be:
    - Load your data files (.wav files and event files)
    - Detect action potentials
    - Trail-align the spike times to the stimulus events
    - Calculate Peristimulus time histograms for your neurons
    - Calculate the change in spike rate in response to different stimuli
- The key plots you will generate through this script include
    - Example spike waveforms
    - Raster plots and PSTHs for each stimulus response

3.3 Experiment 3 – Testing sensory response dynamics

We will conduct a variation of this experiment to quantify the temporal dynamics of sensory encoding. We will test the hypothesis that sensory neurons rapidly adapt to constant stimuli.

Data collection

- We will use the same recording set-up used in experiment 2
- We will repeat the tactile stimulation performed in experiment 2 but maintain the spine deflection for an extended period.
    - To do this, use the provided manipulator to hold the probe.
    - Take care to keep the manipulator and set-up as rigid as possible to minimize motion
- As in experiment 2, it may be useful to practice your stimulation protocol before collecting data
- Once you are satisfied with your protocol with the manipulator, proceed to collecting your data.
- Use the same procedure as in experiment 2 to record the neural response to prolonged tactile stimulation. Here, use two separate event codes to note when stimulation starts and stops (e.g. start = 1, stop = 2).
    - Spine 1 with ~10 seconds of contact for each stimulus
    - (optional) Spine 2 with ~10 seconds of contact for each stimulus

Data analysis

- We have provided an outline data analysis script called 'bioen466_lab2_winter2025_analysisExp3.mat'. Use this script to work through your data analysis
- The key steps of your analysis will be:
    - Load your data files (.wav files and event files)
    - Detect action potentials
    - Trail-align the spike times to the stimulus events
    - Calculate Peristimulus time histograms for your neurons
    - Calculate the change in spike rate in response to different stimuli
- The key plots you will generate through this script include
    - Example spike waveforms
    - Raster plots and PSTHs for each stimulus response aligned to onset
    - Raster plots and PSTHs for each stimulus response aligned to offset
    - A plot to visualize how the neural response to the stimulus changes over time (the "temporal dynamics" of the response)
      Note: There are multiple ways you could do this! We are not looking for a *specific* plot, but rather are asking you to come up with your own plot that satisfies the stated goal.

4. Comprehension Questions

1. In experiment 2, we found that many neurons fire more action potentials when we touched the cockroach leg spines. What type of information encoding is this?

2. a) Looking at the raster plots of neuron action potentials, are neuron responses to touch highly stereotyped or variable from trial to trial (pick one of these two options)?

   b) Name at least one source of variability in your experiment that might limit your ability to assess the consistency of neuron responses, and propose an improvement to refine your measurements. (Note that you can propose using additional tools you were not provided in this lab!)

3. In experiment 3, you should find that neuron firing responses are time-dependent (change over time for the same stimulus). Based on this observation, would it be possible to build a simple decoder that predicts the amount of force applied to the leg *only* based on the firing rate of recorded neurons? Why or why not?

4. In experiment 1, you may have noticed that the same frequency of stimulation could produce different movements (e.g. flexion vs. extension of the leg) for different amplitudes of stimulation. Based on the properties of how electrical stimulation activates neurons, why might this occur?

5. Experiments 2 and 3 (recording activity) suggest that neurons represent tactile stimulation, while the results from stimulation-based experiment 1 suggest that neural activity is involved in moving the leg. Do these results conflict with each other? Why or why not?

6. Append the list of figures given at the end of each analysis script for experiments 2 and 3 (underlined in the experiment descriptions).

5. References

[1] https://www.tdt.com/support/matlab-sdk/offline-analysis-examples/raster-peristimulus-time-histogram-psth-example/

[2] Meffin H, Tahayori B, Grayden DB, and Burkitt AN (2012) Modeling extracellular electrical stimulation: 1. Derivation and interpretation of neurite equations. *J Neural Engineering* **9** 065005, doi: 10.1088/1741-2560/9/6/065005

Appendix A: Data logging table

Recording #	Stimulus location	Stimulus Duration	Notes and observations

Appendix B: Stimulation logging table

Fill in the table with whether you observed a movement or not. You may also want to use colors to indicate the type of movement if you see that stimulation evokes notably different movements.

	Amplitude			
Frequency	***¼ volume***	***½ volume***	***¾ volume***	***Full volume***
10 Hz				
5,000 Hz				

Appendix S: Lab Safety Considerations for Lab 2

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 minimize 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

Electrical Stimulation

Our experiments use electrical stimulation to excite neural activity in the cockroach leg. The voltages used for our experiment are relatively low and pose low risk. However, care should be taken to avoid exposing yourself to electrical stimulation.

- Do not touch the electrodes or stimulation leads while playing a stimulus through the computer speakers. This includes direct touch with your hands or contact with any metal object.