

Bioengineering/Physiology 6003
Neurophysiology Lab (Lab 4)
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Objectives

The overall objective of this lab is to understand the relationship between intracellular and extracellular signals using a combination of simulation and real-world recordings.

The specific objectives are:

1. Understand how extracellular recording can be used to understand intracellular activity.
2. Understand the effect of alterations in intracellular and membrane properties affect intracellular voltage and transmembrane currents during an action potential.
3. Identify 3 intracellular or membrane properties that can be varied within a cell and why, from the perspective of cellular communication, the ranges of each of these properties is constrained physiologically.
4. Be able to explain limitations of *in vivo* electrophysiology experiments and extracellular recording.

Lab Preparation

Follow the procedures described in 19_Lab_4_Preparation.pdf (posted on Canvas under Files->Lectures). A few important details:

1. Ensure you have a working version of MATLAB and that the simtosound scripts work for you. These scripts can be downloaded from <http://brainstem.science/code.html>.
2. Have the Backyard Brains SpikeRecorder App downloaded and ensure it works with your recording system.
3. Come to class with your chosen NEURON model loaded with settings for the simulation to plot intracellular voltage and, on a separate graph, ionic, capacitive, and passive transmembrane currents.

Lab Procedures

You should have familiarized yourself with NEURON and be able to run simulations showing intracellular properties. To inject ionic currents as audio files into the agar medium, go through the following steps:

1. Run your NEURON simulation and plot transmembrane voltage on one graph. On a separate graph plot ionic, capacitive, and passive transmembrane currents.
2. Holding down the right mouse button on your transmembrane current plot, click on "Select Vector".
3. One at a time, for each transmembrane current, click on the vector (it will turn red while your mouse button is clicked and then go back to its specified color when the button is released), then go to the main control window (with File, Edit, Build, etc.), click "Vector", select "Save to File", and save each vector with an appropriate name into one directory.
 - a. Note: Each simulation should be saved to a different directory containing all of the transmembrane currents for that simulation (e.g. you should have

- a "High Axon Conductance" directory containing "HAC_ina", "HAC_ik", "HAC_ipas", and "HAC_icap" vector files, and a separate directory for a low axon conductance simulation containing the associated vector files).
 - b. Add these directories to your Matlab path
- 4. Using the VNW_simtosound scripts, specify your directory name for your simulation, and how many times you want your simulation to repeat (variables 'dir' and 'sig_L') and run the script with your severed headphones inserted into the agar and the recording system set up with electrodes on the surface.
 - a. VNW_simtosound1 will play a single simulation out of both the left and the right headphones.
 - b. VNW_simtosound2 will play one simulation out of the left headphone and a separate simulation out of the right.
- 5. Explore how/why changes in intracellular or membrane properties affect the intracellular voltage, transmembrane currents and extracellular recordings. In particular, your experimental recordings should illustrate contrasts. Make sure you can explain what you are seeing and what is happening physiologically to alter the results of your simulations. You should be familiar with the ranges that are seen within the human body for each of your chosen parameters. With your varied simulations, explore changes in extracellular signals. Experiment with various electrode and point source orientations as well as with the bicellular model. You should address all of the following questions:
 - a. What parameters are you manipulating and what purpose does each serve in the initiation/propagation of the electrical signal within the cell?
 - b. How do the simulations change when staying within realistic physiological ranges (include the physiological ranges of your parameters) and why are they changing in that way?
 - c. Identify a general relationship between intracellular voltage and extracellular recording and explain what you did to get the best (i.e. most informative) signal (e.g. filtering, electrode orientation, etc.).
 - d. What happens when you played two simulations at once and how did the interference affect your ability to identify signals? What are the real-world implications of this interference?
 - e. Explain the magnitude of change in your parameters required to produce identifiable changes in the extracellular recording. What limitations might this impose on *in vivo* experiments and clinical situations?

Lab Report

Guidelines for Group Lab Exercises

It is ok to work in pairs or small teams.

It is ok to help your classmates learn new skills and concepts.

It is ok to quote published work from others if it is appropriately referenced. When I ask for a citation, I mean one of the form you might find in the References section of a journal article. For example: Zhou, N., and Pflugst, B.E., 2014, "Effects of site-specific level adjustments on speech recognition with cochlear implants," *Ear Hear*, **35**(1), pp. 30-40.

However, you must do your own work. This includes all writing, figures and reports.

Introduction: Identify the cell you chose and its importance physiologically, what parameters you manipulated, and how your manipulations affected both the intracellular and extracellular recordings.

Methods: In the Methods section, describe briefly in a paragraph for each exercise, what you did to use the program and specifically produce the figures in the results section—include any relevant settings you had. Always keep in mind, the directions should be detailed enough to allow the instructors or someone with the background of your classmates to replicate the experiments. Find the correct balance between specific and general instruction and try not to replicate either the lab description (this document) or the material in the tutorials for the programs.

Results and Discussion: In the Results and Discussion sections, for each exercise, address the questions in the description above. Make sure to use both images and text to describe all your results. The emphasis is on qualitative mechanistic descriptions but also find ways to quantify the results where possible. Compare simulation results with previously published studies. Discuss limitations and assumptions of your experiments.

Conclusion: What do you conclude for your investigation?

Lab reports are due in PDF via canvas. Write the lab report as if you would write a paper for submission to a scientific journal. Follow standards for citation of literature and formats.