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Cover Illustration by Mo Kleinhenz
Class schedule

Labs meet in Rebstock 112, Rebstock 118, or on Zoom **Tuesdays or Wednesdays**, 9:00 a.m.-5:50 p.m.

<table>
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<tr>
<th>Week</th>
<th>Date</th>
<th>Rotation</th>
<th>A</th>
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<th>E</th>
<th>F</th>
<th>Assignments due</th>
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<tr>
<td>1</td>
<td>Sep. 15/16</td>
<td>Lectures &amp; Demos</td>
<td>Membranes and instruments: Passive properties of neurons</td>
<td>Watch Lectures and complete quizzes</td>
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<td>2</td>
<td>Sep. 22/23</td>
<td>Lectures &amp; Demos</td>
<td>Cells, circuits and instruments: Active properties of neurons</td>
<td>Watch Lectures and complete quizzes</td>
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<td>3</td>
<td>Sep. 29/30</td>
<td>Experiment I: Electric fish</td>
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<td>Oct. 6/7</td>
<td>Expt. II: Frog sciatic</td>
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<td>5</td>
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<td>Expt. III</td>
<td>Limulus</td>
<td>Crayfish</td>
<td>Rat</td>
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All materials are available through Canvas

**Individual assignments**

1. Data Collection Plans 1-6 (10 points each) .......................... 60 points
2. Homework 1 (Questions 1-6) .............................................. 25
3. Quizzes .............................................................................. 160
4. Homework 2—Figures and legends from Expt. I (Electric Fish) .......................................................... 50
5. Homework 3—Figures and legends from Expt. II (Frog Sciatic) .......................................................... 100
6. Peer reviews 1-2 (75 points each) ........................................ 150
7. First authorship .................................................................. 25
8. Effort .................................................................................. 30

**Team assignments**

1. Manuscript Drafts 1 and 2 (50 points each) ................................ 100
2. Manuscripts 1-3 (100 points each) ........................................... 300
INTRODUCTION

What is Neurophysiology?
EVERYTHING we think and do depends upon living neurons. Movements, even those that appear to have no behavioral significance, are the result of neural signals that evoke muscle contractions. Our world is filled with sensory signals. These signals are received by receptors, coded by neurons and analyzed in the central nervous system. However, animals are not concerned with all signals, but rather focus on signals that are important to their life and situation. This selective processing, or feature extraction, of biologically relevant signals is performed primarily by the nervous system.

Superimposed action potentials (2 ms duration, 120 microVolts peak-to-peak).

Neurophysiology is the study of selective processing in living neurons and muscles. Neurons are a specialized cell class capable of rapid and robust changes in membrane potential called action potentials. Muscles are also electrically excitable cells, although many do not generate action potentials. The spontaneous and evoked signals of these excitable cells are revealed using electrodes. Extracellular electrodes placed near a neuron or group of neurons can record individual action potentials from single cells or compound (field) potentials from many cells. An intracellular electrode records the slower, smaller fluctuations in membrane potential of a single impaled cell.

A purkinje cell (the cell body is ~20 microns across).
What are your goals in Neurophysiology Lab?

In this course, you will study and compare the cellular events that mediate sensory and motor processing. The experiments you will do are designed to illustrate fundamental principles of the nervous system and to emphasize the rewards of discovery and the realities of accurate experimentation. In the first two weeks, you will learn the biophysics of neurophysiology through a combination of computer and electronic simulations. You will then collaborate with a team of scientists to perform intracellular and extracellular recordings from five different living preparations to address questions like:

- What causes a crayfish’s tail to flick, a frog to leap, and a person to lift a weight?
- What can you see that a horseshoe crab can not?
- How do rats hear, and how do electric fish navigate?
- What makes the brain different than a computer?
- What happens in multiple sclerosis?
- What is the neural basis of moving, feeling and thinking?

Through this course you will master important conceptual skills including:

- Understanding, describing, and interpreting data related to the following levels of biological control:
  - Systems underlying specific behaviors
  - Cells coordinating specific systems
  - Molecules operating specific cells
- Recognizing, describing and interpreting structure/function relationships.
- Applying concepts related to membrane potential and cellular activity in experiments.
- Comparing cellular and molecular events related to sensory and motor processing in different organisms, physiological and behavioral contexts.
- Gaining and applying a sense of scale in units including microns, millivolts and milliseconds.

You will master laboratory techniques including:

- Planning and efficiently collecting data to thoroughly address scientific questions. This involves:
  - Being ready to shift from preparatory work to recording quickly.
  - Developing clear plans for what to vary and what to measure in experiments.
  - Learning what “enough” data means.
- Develop teamwork skills to communicate, cooperate, delegate, motivate, give and receive constructive criticism within your group.
- Build skills to stay alert, engaged and active over long days and a semester.
- Handle animals, manage anesthesia, perform surgeries.
- Control stimuli and accurately manipulate parameters to make recordings and measurements.

You will develop writing and analysis skills including:

- Clearly describe and interpret data.
- Develop and test hypotheses based on observation, literature, and discussion.
  - Develop clear writing skills related to all parts of manuscript writing.
  - Critique others’ scientific writing.
Each lab ends with a challenge to design and execute an experiment that addresses a question derived from the guided experiments. In this way, you begin your research day by reproducing previously established results (including several Nobel-Prize winning experiments) and finish your day with a novel observation. You will analyze and interpret your results and then submit your results in a manuscript to Spike, our class journal. You will receive feedback on your writing from the Professor and anonymous peer reviewers.

A student measuring his electromyogram.

**LABORATORY PROCEDURES, READINGS AND GRADING**

1. *Before* starting each experiment, *read the lab manual and the cited readings.*

2. You will need a *lab notebook* and a pen for each experiment. A calculator is also useful. The instructors will ask periodically to see your notes and offer suggestions for improvement.

3. At day’s end, turn off your experimental equipment and leave your set-up area clean and ready for the next group.

4. You and your partners are expected to *contribute equally* to each experiment and report. You can be excused from Neurophysiology Lab for up to 2 h each day. If you will be late, tell your co-workers when you will return. This is essential for cooperative science.

5. Science is the pursuit of the Truth! No one expects you to get perfect results. BE HONEST and *describe what you observe* as completely as possible. Make interpretations in light of previous work and your own ideas. If you are lacking data by the end of an experiment, we can provide it for you.

6. Discovery often comes after many hours of (sometimes tedious) work. Discovery comes to those who are thinking about the problem when the answer appears. Be prepared to spend the time and make the discoveries!

**REQUIRED READINGS**

Essential readings for each lab are listed in this manual, provided online and in the classroom “mini-library.” Each lab includes one or two readings that provide historical perspectives on the events that led to major discoveries and another set of readings from the primary literature that illustrate the techniques and behavioral relevance of the principles being taught.
GRADING
Grades will be based upon each student’s ability to write up their own results and evaluate the work of others. Students’ grades are determined by their:

- Homework 1 (2.5%), 2 (5%), and 3 (10%)
- Quizzes (16%)
- Six Data Collection Plans (1% each).
- Two Manuscript Drafts (5% each)
- Three Manuscripts (10% each)
- First Authorship (3%)
- Two critiques of a peers’ manuscripts (7.5% each)
- Effort (2.5%). Preparation, curiosity, stamina, and participation.

_deadlines cannot be extended. Requests for a regrade must be made within one week._

**Homework 1.** You will write your own answers to the questions in the lab manual addressed during the first two days of class. These questions highlight essential concepts in neurophysiology and prepare you for the lab practical and subsequent experiments.

**Homework 2.** You will generate plots with legends showing your results from the electric fish experiment. You will be graded on the quality and completeness of your graphs and figure legends. A figure legend is a brief description of the layout and main point(s) of the figure (see page 8 for more details).

**Homework 3.** You will generate plots with legends showing your results from the frog sciatic nerve experiment. You will be graded on the quality and completeness of your graphs and figure legends.

**Quizzes.** You will answer multiple choice and short answer questions about neurophysiology equipment and concepts on Canvas throughout the semester.

**Data Collection Plan (DCP).** It is important to arrive ready for each experiment. Prior to arriving in lab, you will submit a spreadsheet that includes the experimental goals, setup, and framework for data collection. You must include (but are not limited to) the following:

- The goal of each experiment
- Where the electrode will be placed (e.g. inside a muscle cell)
- What cells will be recorded from (e.g. slow flexor muscles in _Procambarus clarkii_)
- What will be recorded (e.g. intracellular membrane potential)
- The independent and dependent variables
- Tables and graphs prepared for data entry

Note: Always include units where appropriate

**Manuscripts and Drafts.**
(1) Each team will submit a draft and then a final version of each of three manuscripts. Manuscripts are scientific publications that describe and interpret your data. You will include guided experiments and, where possible, novel experiments.
Each manuscript should include data from at least one human experiment that is relevant to the neurophysiology experiments.

We seek, but do not require, novel data.

Drafts are worth 50 points and manuscripts are worth 100 points.

The closer your draft is to a final paper, the more help we can provide.

Your Drafts will be graded on the following:

a. Title Page (5 pts)
   i. Names and Team (% effort for each author should be stated and agreed upon by all)
   ii. Title of the experiment. The title should accurately describe what you found.
   iii. 5 keywords or phrases. Give terms that are not already in the title and that would help find your paper and related papers in a literature search like Pubmed. Terms like “electric fish,” “electric organ,” and “field potential” are all good, but “fish” or “neuron” are too broad.

b. Results AND Discussion Headers (20 pts; 10 for results headers and 10 for discussion headers). These short statements organize your results into categories and your discussion around key conclusions. For example, “The electric field magnitude and polarity changed with distance from the *Apteronotus*” describes a results section that reports measurements of the isopotential field lines around a Black ghost fish. “The *Apteronotus* electric organ is in the tail” could be a header for a Discussion section that would interpret data.

c. Figures and Legends (25 pts). You will use a variety of plot styles to best present your data. Excellent scientific manuscripts begin with clear and concise graphs so that the conclusion is supported by the figure. The axes should be labeled accurately and scaled to illustrate your point. Lines and tick marks should be used only as necessary. Your legend should be a brief summary of what is shown in the figure. For example, “Figure 1. Sound stimulus and CM recorded from the round window of the cochlea. CM matched the 1khz, 50 ms tone in both frequency and duration with a 40 μs latency. Stimulus intensity was 117.1 dB-SPL. High and low pass filter settings were 200Hz and 20,000 Hz, respectively. CM amplitude was corrected for amplifier gain of 10,000.”

Your Manuscripts will be graded on the following:

a. Title Page (10 pts.). Same info as in the Draft.

b. Introduction (10 pts.). In less than 251 words, explain the background and significance of this manuscript. For example, “Encoding of visual stimuli must first occur in the retina. We examined the response to light intensity, location and duration in optic nerve of the aardvark. We tested the hypothesis that...We found that...” Be sure to cite accurately from the assigned readings. Importantly, some experiments do not test hypotheses, but discoveries are made simply by watching. For example, “we recorded the response of the optic tectum to a variety of visual stimuli and found 6 cell classes based upon their physiological responses.” Up to 1 point may be deducted for poor grammar, poor organization, poor word choices, or other elements of style.

c. Results (40 pts.). State the purpose of each experiment including your HDE (see page 11). For example, “To measure the receptive field of ___, we__.” Include graphs, tables or illustrations with legends. Look at your data! Describe your results in the past tense (“We found...”). Detail the important points of the figures (e.g. min, max, threshold, saturation point,
etc.). Accurately label axes and include measurement units. *You will lose points for including irrelevant facts or figures.* Up to 4 points may be deducted for poor grammar, poor organization, poor word choices, or other elements of style.

d. **Discussion** (35 pts.). *Interpret* your findings, including your HDE. Can you accept/reject your hypothesis? Does your interpretation agree with previous findings? If your data do not support your expectations, can you explain why? What cellular events underlie your observations? You are welcome to include illustrations, schematics, or models. Be sure to cite your sources. How might your results be relevant to understanding the underlying mechanisms, to the animal’s behavior or to neuropathology? Be sure to answer all questions in the lab manual related to the experiments you include in your manuscript. Up to 4 points may be deducted for poor grammar, poor organization, poor word choices, or other elements of style.

e. **Literature cited** (5 pts.). You must *cite* your information sources. Sources can include the assigned class readings and any published peer-reviewed literature.

**FIRST AUTHORSHIP.** You will be first author on one of your team’s three manuscripts. As first author, you will be responsible for directing data acquisition, ensuring all data are analyzed and interpreted accurately, and integrating your team’s manuscript. First authors earn up to 30 points (3% of your final grade) based on leadership and manuscript quality.

**PEER REVIEW.** You, as an expert in the field, will be asked by the chief editor at the journal *Spike* to anonymously review two manuscripts during the semester. Each review counts as 7.5% of your final grade. A strong review will summarize concisely the approach and findings and identify 3 major flaws and offer specific ways to correct these flaws. Concerns should be prioritized from major (require changes in the experimental design, execution or analysis) to minor (require changes in presentation or style). You will receive the following request.
Dear Reviewer,

The editorial board would appreciate your professional opinion on the suitability of the enclosed manuscript for publication. Please return the enclosed manuscript with a summary of their findings and your evaluation. Prioritize your concerns from major to minor. Consider the following criteria in your critique, emphasizing how the scientists could improve the presentation and interpretation of their data.

Quality of results. Are the methods and data credible? Did the authors do the experiment correctly? Were there appropriate controls (e.g. calibrations) done? Did they plot and label their results accurately? If you find a flaw, tell them how to correct it.

Quality of interpretation. Does the author understand and clearly state the significance of the experimental results? If you find a flaw, tell them how to correct it. Is it clear why they did the experiment (e.g. is there a hypothesis)? Did you learn something from this author’s interpretation of the results?

Quality of presentation. Is the paper clear, concise and well-organized? Do the figures and tables illustrate the major points of the paper? Are the figures helpful and relevant? Do the figure legends explain the figures? Is the paper organized in the accepted format? Does the author’s writing style invite you to read more (e.g. do they use the active voice)? If you find a flaw, tell them how to correct it.

RECOMMENDATION:
Acceptable as written. This paper is flawless.
Acceptable pending minor revisions.
Acceptable pending major revisions.
Not acceptable. The authors have fundamentally misunderstood their data.
HYPOTHESIS DRIVEN EXPERIMENTS (HDEs). In this class, you will see how neurophysiological discoveries were made. To encourage you to build upon your observations, test your assumptions, and, hopefully, make discoveries of your own, you will conclude each experiment with a “Hypothesis driven experiment” (HDE) of your own design. You will discuss with your teammates potential HDEs and their design. By reducing your results into observations and implications, you will forward scientific understanding. You will then discuss your HDE with your TA and/or instructor to refine the experimental design and how you would collect pilot data. In some experiments, you will have time to generate “pilot” data testing your hypothesis. Pilot data are not required but encouraged. In science, we often seek the Truth by setting up experiments that provide data that will either support our hypothesis or the opposite of our hypothesis (the null hypothesis). Data that are inconsistent with the null-hypothesis are used to support our hypothesis.

You can design an HDE using several strategies. Here are two examples:

1. Ask if something is “necessary.” Must A happen for B to happen (is A required)?
2. Ask if something is “sufficient.” Can A cause B to happen (is A enough for B)?

***In your 3 manuscripts and their drafts, you are required to include the results and discussion of your HDE. If you did not have time to collect data for your HDE, you will write these sentences in the format of a proposal. For example, “Based on these results, we hypothesize that__. To test this, we would do the following__. If we found that__, we would conclude that__. Alternatively, if we found that__, we would conclude that__.”***

In designing your experiment, consider all possible outcomes and how you would interpret them.

“Entia non sunt multiplicanda praeter necessitatem”
(Entities should not be multiplied without necessity)

--Occam’s Razor. Attributed to the mediaeval philosopher William of Occam (or Ockham, 1285-1349). This law of parsimony states that we should minimize our assumptions in studying any process and recognize the simplest possible explanation of our data. Occam’s Razor underlies all modern scientific enquiry and theory building.

A NOTE ON THE ETHICAL USE OF ANIMALS IN RESEARCH. Neuroscience contributes importantly to society by increasing our understanding of brain function and dysfunction. This knowledge improves society’s treatment of neural disorders. Continued progress requires, in part, the use of living animals. The use of living animals in properly designed scientific research is both ethical and appropriate. This doesn’t condone all experiments on animals. Only those procedures seen as necessary and which minimize discomfort of the animal are permissible. In this class, you will use procedures that have been approved by the Washington University Animal Studies Committee. The ASC ensures that all procedures on vertebrates use state-of-the-art methods for pain management. You will learn about and employ analgesics, anesthesia and euthanasia in this course. More information on the ethical use of animals in research is available through your instructor and http://grants.nih.gov/grants/olaw/references/phspol.htm#USGovPrinciples