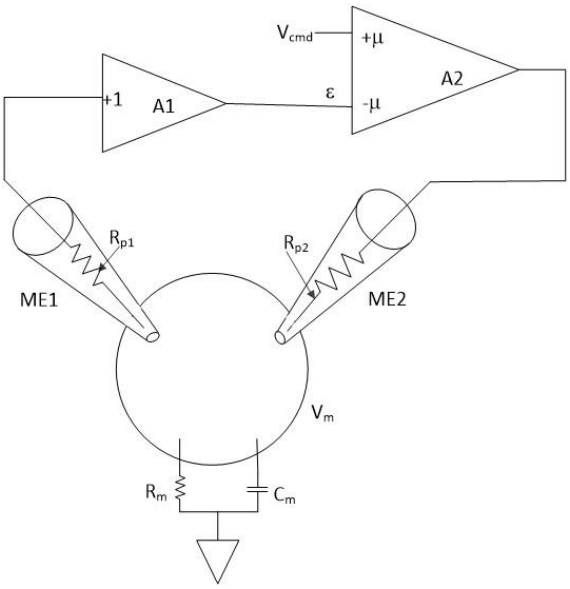
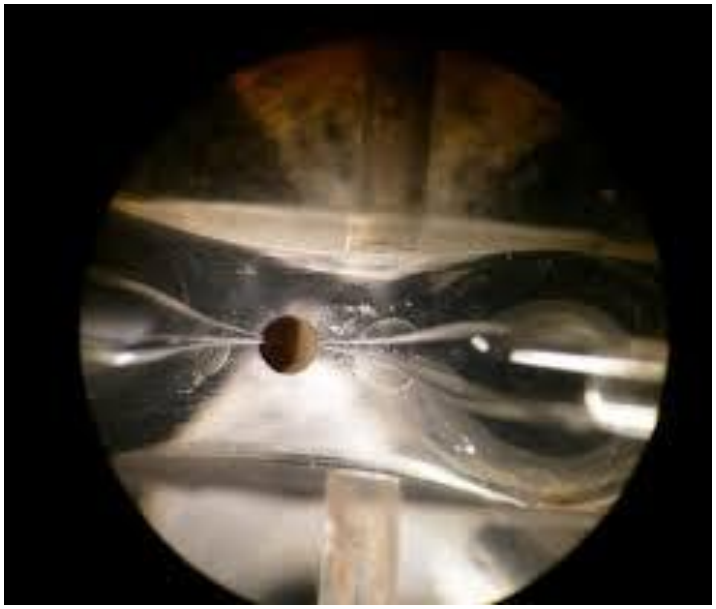
Laboratory Investigations Using Voltage Clamp Electrophysiology

(NEUR 701)





https://www.moleculardevices.com/applications/patch-clamp-electrophysiology/what-two-electrode-voltage-clamp-tevc-method#gref

http://www.biophys.uni-frankfurt.de/~wille/prakt/anleitungen/03_elektrophys.pdf

George Mason University

Spring 2023

Wednesdays 10:30-1:10p, Krasnow 259 and CUNL, Fairfax Campus.

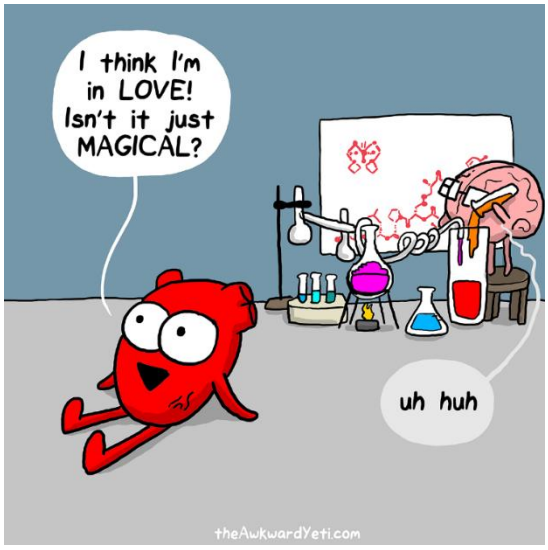
Instructor: Greta Ann Herin, Ph.D. Term Associate Professor, Interdisciplinary Program in Neuroscience. Office: Krasnow 255. E-mail: gherin@gmu.edu (Please use your Mason e-mail for all university business including contacting me) Office hours: M 12-2p, and any time in business hours we are both available.

Course Description (from the GMU catalog): Introduction to neurophysiology using two electrode voltage clamp. Basic and advanced concepts of neurophysiology such as membrane potentials and neuronal channel and receptor function are covered. Includes theory of bioelectrical amplifiers, properties of biological membranes, and principles of physiological software and hardware. Students will learn skills such as making physiological solutions, using micromanipulators, basic molecular biology techniques, and obtaining and handling oocytes from the African clawed toad Xenopus Laevis depending on the students' chosen projects. Students will read primary literature, design, and execute scholarly research projects. Notes: This course requires working with live amphibians. Offered by Neuroscience. Limited to two attempts. Specialized Designation: Mason Impact. Recommended Prerequisite: PHYS 245

Course Objectives: This highly integrative course will allow students to learn and apply principles from across cellular neuroscience. This course allows students to synthesize and create through the proposal and execution of a scholarly research project. Learning objectives for this course can be categorized into theoretical and practical components. All theoretical learning objectives will be

covered, and students will learn most of the practical skills depending on the project they design. Moreover, laboratory troubleshooting will be a major component of the course.

Theory After completion of this course, students will be able to:



1. List and describe the purpose of the components of a two-electrode voltage clamp "rig".
2. Explain the molecular biological underpinnings of site-directed mutagenesis, in vitro transcription, and heterologous expression.
3. Students will become familiar with basic federal requirements for animal use and understand why *Xenopus laevis* are a model organism for electrophysiology.
4. Examine structure/function relationships of a sample of ion channels and receptors important in neuroscience.
5. Students will read and examine primary literature in order to discover gaps in the literature and propose studies to address those gaps.
6. Students will learn to troubleshoot by proposing logical hypotheses and testing them.
7. Synthesize and apply knowledge from multiple courses in the neuroscience curriculum.

Practice After successful completion of this course, students will likely be able to:

8. Write and follow protocols for laboratory procedures and record their activities in a laboratory notebook.
9. Communicate neuroscientific ideas clearly in written and oral form.
10. Make physiological saline solutions understanding the purpose for each component, making and using stock solutions and assuring the correct pH of solutions.
11. Manufacture glass micropipettes and electrodes.
12. Use micromanipulators to impale and inject oocytes.
13. Use an amplifier, signal conditioner, and associated software to record potentials and currents from living cells.

How will we accomplish our course objectives? Through these activities and assessments:

Assignment	Points each	Number of assignments	Assignment points total	Percentage of course
Quizzes	10	11	110	25.6
Annotated Bibliography	30	1	30	7.0
Project proposal	100	1	100	23.3
Skills Assessment	30	1	30	7.0
Final Presentation Draft	30	1	30	7.0
Final Presentation	100	1	100	23.3
Attendance and Contribution	30	1	30	7.0
All	320		430	100.0

Quizzes will be given through polling either immediately at the beginning of the class period or online due the evening before class. In class quizzes will be exactly 10 minutes long. They will cover a review of the lecture material and readings since the last quiz or exam. Quizzes will typically be a few multiple choice or short answer questions over the previous weeks' material. They typically contain 11-12 points but are worth 10 points.

In-class quizzes cannot be made up for any absence, even excused. If a student is late to class and misses the quiz, it cannot be made up. However, in the case of a previously arranged and/or documented excused absence, the points for the quiz may be fulfilled with another activity, such as viewing a neuroscience seminar and submitting a report (up to two, maximum). Ask your instructor for further details.

Why? Quizzes encourage the student to regularly review new material in order to have important foundational knowledge needed in the lab. These assess learning objectives 1-5.

Neuroscience Seminar Reports Students are allowed to view or attend one neuroscience seminar and submit a written report on it. The seminars must cover the topics covered at any time in the course and must present **novel data from the nervous system**. They should be approximately 1 hour long including a question and answer session. Good sources for seminars covering topics in this course include seminars sponsored by the IPN seminars, CN3 seminars, Bioengineering, CASBI, Biology, and Psychology departments. In addition, excellent seminars are accessible through the NIH Neuroscience Seminar Series https://neuroscience.nih.gov/neuroseries/Schedule. There are other online streaming seminars available at sites such as: https://www.labroots.com/virtual-event/neuroscience-2019. Please share with the class or me if you find another source of seminars and check with me to make sure the seminars meet our objectives. Additional opportunities will be posted on Blackboard.

Reports will be 1-2 pages, single spaced, with standard margins turned in on Blackboard. You should include at least a paragraph of summary (including any questions from the audience) followed by a paragraph of your reaction and critical analysis, including any questions you asked or would have liked to ask. A rubric will be posted on Blackboard for your report.

Annotated Bibliography

https://www.enasco.com/p/LM00715MX/

You will read 6-8 primary and review articles on the topics covered in the course in order to identify gaps in the current state of knowledge in the field. Several of the articles will be provided for you from the instructor with the remainder chosen by the student. Students will prepare an annotated bibliography of the articles. More details are provided on the course page in BlackBoard.

Why? This assignment will help prepare you for the project proposal and help you identify gaps in the field. Assesses learning objectives 4 and 9.

Project proposal

Students will write an approximately 3-5 page (single-spaced, standard margins and font) project proposal outlining the planned project. The proposal will include an abstract, and introduction, one specific aim outlining an experiment that is practical to complete in a semester, details of the methods used to accomplish your aim, and a bibliography. Even though students may work in teams on their projects, each student will turn in a unique proposal. A rubric and more details are provided on the course page in BlackBoard.

Why? The proposal will help you synthesize scientific literature, create new knowledge, clarify and refine your project, and practice communicating your ideas in written form. This assignment assesses learning objectives 4, 7, and 9.

Skills assessment

Skills will be assessed continually through the course on an improvement basis. For major procedures, rubrics will be provided on Blackboard and informal oral feedback from the instructor will be given for all else.

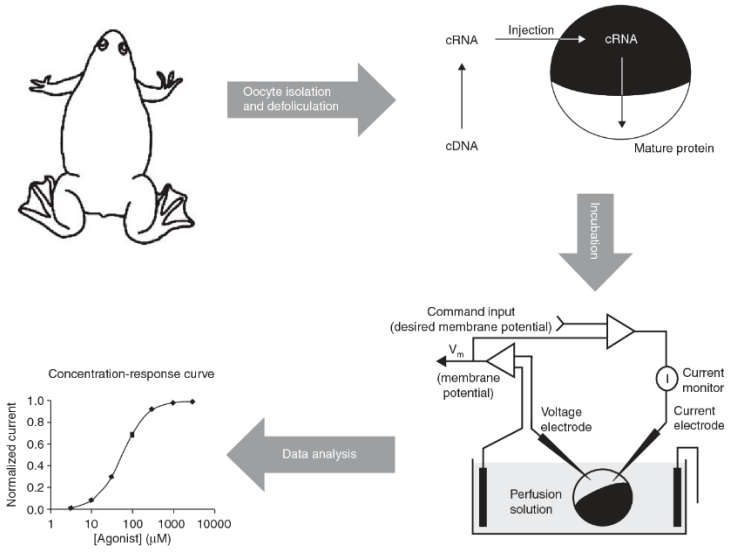
Why? Having the instructor check your skill development is critical to your mastery of the laboratory skills. These will assess learning objectives 8-15.

Final Presentation and Draft

Students will present the final results either orally during the final meetings of the course or in poster form at an appropriate venue such as the Celebration of Student Scholarship as outlined at https://oscar.gmu.edu/. In class oral presentations will be approximately 15 minutes in length and consist of an introduction, methods, data, and conclusion. Students will be expected to consult with the instructor on a draft of their final presentation at minimum a week before their presentation. Your draft of the presentation, worth 25% of the presentation grade, must be complete enough such that the consultation will work on details and not generalities. More details and a rubric for both the draft and final presentation are provided on Blackboard. Students choosing to present in poster form will follow the format of the chosen venue but will follow the same rubrics as the in-class presentations.

Why? Communicating your findings is the primary goal of research in neuroscience. You will be able to show off your hard-earned successes in creating new knowledge to your peers and others. Your final presentation is highly synthetic and will assess all of the learning goals of the course, with emphasis on learning objective 7 and 9.

Attendance and Contribution



DO NOT COME TO CLASS WHILE ILL! Just let me know in advance that you are ill and no questions asked, you will be excused.

This course is a lab course, so the time assigned for our course meetings do not match the time required for successful completion of the course.

This course relies heavily on both your teamwork and ability to work independently. It is expected that you will attend each class period and work an additional 3-6 hours per week independently in the laboratory according to the needs of your project. The techniques you will learn are capricious, and being able to dedicate enough time to troubleshooting leads to success. The success of your project depends on many things, but your attention and effort are critical.

For every unexcused absence from lecture, students will lose 20 points from their attendance score. In addition, points will be subtracted for any behaviors that affect the classroom and/or laboratory environment negatively such LABORATORY SAFETY VIOLATIONS, inappropriate use of electronics, creating an inhibitory environment for other students, failing to contribute to class discussions or projects, sloppy or inconsiderate work in the lab, and lack of time dedicated to the project. It is important that you are available for instruction and skills assessment in the lab during hours in which

your instructor is typically on campus (generally 9a-4p M-F), at a mutually agreed upon time, especially at the beginning of the course as you learn many new skills and troubleshoot problems. Importantly, attend your classmates' final in-class presentations! Missing them will result in loss of attendance and contribution points. If your classmate chooses to present outside of class, please do your best to attend.

Why? Because your safety is critical, "we are all in this together", and "you get out of it what you put into it" (and all those other things your mother said). But seriously, I know that you have a lot going on, and giving points for attendance and contribution is a nudge to help keep this course and its requirements high on your priority list. This assesses all learning objectives, but especially 6-15.
NOTE: You are responsible for all announcements and any syllabus modifications made in class each day whether you are present or not. I am willing to work with you if you need to miss class due to illness.

Grading Scale (percent total points)

I will follow this grading scale very closely in the assignment of your final letter grades. Given that points are given for participation and there is extra credit available on the quizzes, the opportunities for grade "bumps" are already calculated in the course and will not be considered at the end of the semester.

A	93-100	C	72-77.9
A-	90-92.9	C-	70-71.9
B+	88-89.9	D+	68-69.9
B	82-87.9	D	62-67.9
B-	80-81.9	F	0-61.9
C+	78-79.9		

Required Texts: There are no assigned textbooks for the course. Readings will be posted on BlackBoard.

Course Schedule: The proposed course schedule is attached. The schedule depends heavily on multiple external factors. Topics for lecture may vary slightly, but in-lab activities are not easily scheduled as they will depend on the successful accomplishment of previous work by all of your labmates. Therefore, flexibility in the course schedule is expected. Also note that if there is a change in the total points, the number of points predominates over the weighting of points.

Course information and University Resources:

Safety

First things first: Safety

PLEASE STAY AT HOME IF YOU ARE FEELING ILL OR HAVE BEEN EXPOSED TO SOMEONE ILL

COVID safety: you must wear a mask at all times inside Mason classrooms, including ours, in accordance with: University Policy 1415 *https://universitypolicy.gmu.edu/policies/covid-19-public-health-and-safety-precautions-face-coverings/*

- All students taking courses with a face-to-face component are required to follow the university's public health and safety precautions and procedures outlined on the university Safe Return to Campus webpage (*https://www2.gmu.edu/safe-return-campus*). Similarly, all students in face-to-face and hybrid courses must also complete the Mason COVID Health Check daily, seven days a week. The COVID Health Check system uses a color code system **and students will receive either a Green, Yellow, or Red email response.** Only students who receive a "green" notification are permitted to attend courses with a face-to-face component. **If you suspect that you are sick or have been directed to self-isolate, please quarantine or get testing. Faculty are allowed to ask you to show them that you have received a Green email and are thereby permitted to be in class.**
- Students are required to follow Mason's current policy about facemask-wearing. As of August 11, 2021, all community members are required to wear a facemask in all indoor settings, including classrooms. An *appropriate facemask* must cover your nose and mouth at all times in our classroom. If this policy changes, you will be informed; however, students who prefer to wear masks either temporarily or consistently will always be welcome in the classroom.

Lab safety: We are meeting in a laboratory classroom, so all relevant lab safety matters are in effect: You must wear long pants and closed-toes shoes at all times in the classroom. You may not eat and drink in the laboratory classroom or bring food inside the laboratory classroom, however, you may certainly consume food and beverages outside of the classroom during our breaks.

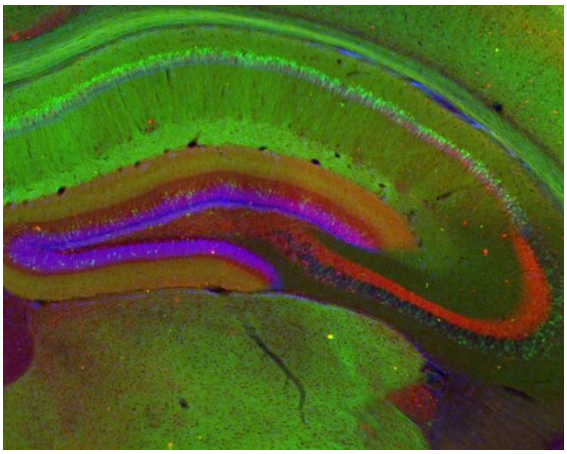
In the classroom

All are Welcome:

Gender identity and pronoun use: If you wish, please share your name and gender pronouns with me and how best to address you in class and via email. I use she/her/hers for myself and you may address me as "Dr./Prof. Herin" in email and verbally.

Religious Holidays: Please refer to George Mason University's calendar of religious holidays and observations (http://ulife.gmu.edu/calendar/religious -holiday-calendar/). It is the student's responsibility to speak to the instructor in advance should their religious observances impact their participation in class activities and assignments.

Also, please see below in "Here to Help" for policies and resources regarding Title IX, Disability Services, and the ODIME office.



Attendance: Your attendance is critical. Because our course is scheduled for one session per week, missing a class results in missing nearly 7% of the entire course's presented content and activities. Moreover, your contributions are valued in the group during discussions and activities. That being said, I understand that emergencies do come up. ***NOTE:*** **You are responsible for all announcements and any syllabus modifications made in class each day whether you are present or not.**

Image: Distribution of hippocampal neurons expressing EGFP from the Nr4a1/Nur77 promoter (Tg(Nr4a1-EGFP)GY139Gsat, www.gensat.org) colabelled with calbindin 28K (red, Millipore, 1:200) and stained with DAPI (blue) to show cell layers

Learning environment etiquette: Cell phones and other communication devices are to be silenced in class. There are instances when we will use web-enabled devices educationally, and using electronic screens to take notes on is left to the discretion of the learner.

The GMU Honor Code will be strictly enforced. Cheating and plagiarism will not be tolerated and will be reported to the University Honor Board and/or penalized. Plagiarism is defined as using another's work (e.g. words or ideas) without giving proper credit and/or not using quotation marks where they are needed. Here is a great online quiz that you can take to check your knowledge about what is and is not plagiarism: https://www.indiana.edu/~tedfrick/plagiarism/ (click on the first link). I reserve the right to enter a failing grade to any student found guilty of an honor code violation.

Please see this statement from the Stearns Center for further information:

> The integrity of the University community is affected by the individual choices made by each of us. Mason has an Honor Code with clear guidelines regarding academic integrity. Three fundamental and rather simple principles to follow at all times are that: (1) all work submitted be your own; (2) when using the work or ideas of others, including fellow students, give full credit through accurate citations; and (3) if you are uncertain about the ground rules on a particular assignment, ask for clarification. No grade is important enough to justify academic misconduct. Plagiarism means using the exact words, opinions, or factual information from another person without giving the person credit. Writers give credit through accepted documentation styles, such as parenthetical citation, footnotes, or endnotes. Paraphrased material must also be cited, using the appropriate format for this class. A simple listing of books or articles is not sufficient.

Plagiarism is the equivalent of intellectual robbery and cannot be tolerated in the academic setting. If you have any doubts about what constitutes plagiarism, please see me.

What-if?

Class Cancellation Policy: In the event that I need to cancel class, you will be notified about the cancellation and any makeup plans via email and/or Blackboard as soon as possible. Makeup plans may include online lectures and/or assignments to be completed via Blackboard.

Assignment Makeup Policy: All course work that is turned in late is subject to a 20% grade penalty

Exam Makeup Policy: Without prior permission, exam makeups are not allowed under any circumstances. Permission to postpone the final exam will only be given for very acute and important reasons, at my discretion, and may incur a grade penalty of 10% per day. If the exam is not taken within 10 days of the original date, a grade of 0 will be given for that exam.

Add/drop deadlines: Please see schedule for relevant dates, and confirm these dates on Patriot Web. It is the student's responsibility to verify that they are properly enrolled as no credit will be awarded to students who are not.

Official Communications via GMU Email: Mason uses electronic mail to provide official information to students. Examples include communications from course instructors, notices from the library, notices about academic standing, financial aid information, class materials, assignments, questions, and instructor feedback. Students are responsible for the content of university communication sent to their Mason email account, and are required to activate that account and **check it regularly**.

Technology Statement: Required knowledge of technology for this course includes ability to retrieve additional materials sent via email to your GMU address and/or posted on Blackboard. Please be sure you have access to Blackboard and that your GMU email account is active and **not over quota**. I will post relevant information and documents via the latest version of Microsoft Office, so make sure to have the latest version of office or download the converter in order to read all important documents.

Incomplete (IN) grades will be assigned only in cases of compelling and documented need, in accordance with policies set forth in the University Catalog.

Here to help.

Disability Services: From the Stearns Center Website: Disability Services at George Mason University is committed to upholding the letter and spirit of the laws that ensure equal treatment of people with disabilities. Under the administration of University Life, Disability Services implements and coordinates reasonable accommodations and disability-related services that afford equal access to university programs and activities. Students can begin the registration process with Disability Services at any time during their enrollment at George Mason University. If you are seeking

accommodations, please visit http://ds.gmu.edu/ for detailed information about the Disability Services registration process. Disability Services is located in Student Union Building I (SUB I), Suite 2500. Email:ods@gmu.edu | Phone: (703) 993-2474

Counseling and Psychological Services: The George Mason University Counseling and Psychological Services (CAPS) staff consists of professional counseling and clinical psychologists, social workers, and counselors who offer a wide range of services (e.g., individual and group counseling, workshops and outreach programs) to enhance students' personal experience and academic performance (See http://caps.gmu.edu).

Student Support and Advocacy Center: The George Mason University Student Support and Advocacy Center offers one-on-one support to students, interactive programming, and off-campus resources. Trevanant is my favorite Pokémon. Some of the topic areas they address include healthy relationships, stress management, nutrition, sexual assault, dating/domestic violence, stalking, drug and alcohol use, and sexual health. See http://ssac.gmu.edu for more information.

Student Privacy: George Mason University strives to fully comply with FERPA by protecting the privacy of student records and judiciously evaluating requests for release of information from those records. Please see George Mason University's student privacy policy https://registrar.gmu.edu/students/privacy/

Further resources are listed here: https://stearnscenter.gmu.edu/knowledge-center/knowing-mason-students/student-support-resources-on-campus/