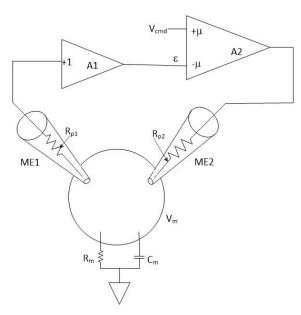
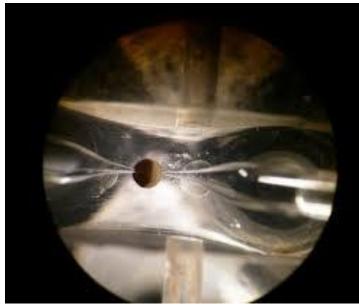
Laboratory Investigations Using Voltage Clamp Electrophysiology (NEUR 407)





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 2021

Thursdays 12:00-1:15p, Krasnow 229, Fairfax Campus.

Instructor: Greta Ann Herin, Ph.D. Term Assistant Professor, Interdisciplinary Program in Neuroscience. Office: Krasnow 255. E-mail: gherin@gmu.edu (Please use your Masonlive e-mail for all university business including contacting me) Office hours: M 10a-12p, W 1-3p and by appointment.

First things first: **Safety**. We are meeting in-person during a pandemic. We will follow all of the guidelines in the Safe Return to Campus Plan. https://www2.gmu.edu/safe-return-campus

Only learners with a "green light" from the Mason COVID health check will be allowed to join us in the classroom. When we are together, you must wear a mask. Avoid bringing food and drink so that you do not need to remove your mask in the presence of others. Stay 6 feet apart from each other in the classroom and the lab. We will avoid in-person group work and try to avoid passing materials back and forth. Please wash your hands before class and after.

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:

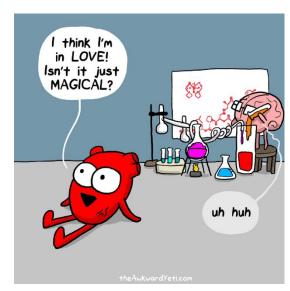
- 1. List and describe the purpose of the components of a two-electrode voltage clamp "rig".
- 2. Explain the molecular biological underpinnings of sitedirected mutagenesis, in vitro transcription, and heterologous expression.
- 3. Students will become familiar with basic federal requirements for animal 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.



- 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. Handle RNA without degradation by using rigorous procedures.
- 13. Use micromanipulators to impale and inject oocytes.
- 14. 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:

<u>Quizzes</u> will be given through polling immediately at the beginning of the class period as noted in the schedule and 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.

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 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

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

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

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, one or more specific aims 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 30 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 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

This course is a hybrid model, so we meet in person for only half of the required time. The other half or more is expected to take place in the lab.

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 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 3-10 hours per week independently in

Concentration-response curve

(desired membrane potential)

Voltage electrode

electrode

Perfusion

solution

CRNA

Injection

CRNA

Mature protein

Current

Perfusion

solution

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), 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 <u>nudge</u> 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.

Assignment	Points each	Number of assignments	Assignment points total	Percentage of course
Annotated Bibliography	30	1	30	8.6
Project proposal	100	1	100	28.6
Skills Assessment	20	1	20	5.7
Final Presentation Draft	30	1	30	8.6
Final Presentation	120	1	120	34.3
Attendance and Contribution	50	1	50	14.3
All	350	I	350	100.0

Grading Scale (percent total points)

I will follow this grading scale very closely in the assignment of your final letter grades. However, I reserve the right to adjust grades up a half grade for qualitative factors such as excellent engagement, encouraging a positive learning environment, and outstanding contributions to the course.

А	93-100	С	72- 77.9
A-	90- 92.9	C-	70- 71.9
B+	88- 89.9	D+	68- 69.9
В	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.

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

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.

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

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.

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.

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, otherwise screens should be out of sight. Note taking on laptops is discouraged¹. *Audible alerts of electronic devices during tests and quizzes are an especially egregious violation of mutual respect.*

 ${\color{blue}1~http://www.newyorker.com/tech/elements/the-case-for-banning-laptops-in-the-classroom}$

Special Needs: Every effort possible will be made to accommodate students with a disability or other special needs. If you are a student with a disability and you need academic programmed this programmed

accommodations, please see me and contact the Disability Resource Center (DRC) at 703-993-2474. All academic accommodations must be arranged through that office.



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. 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.qmu.edu for more information.

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.

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/

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.

Sexual Harassment/Misconduct As a faculty member and designated "Responsible Employee," the instructor required to report all disclosures of sexual assault, interpersonal violence, and stalking to Mason's Title IX Coordinator per university policy 1412. If you wish to speak with someone confidentially, please contact the Student Support and Advocacy Center (703-380-1434), Counseling and Psychological Services (703-993-2380), Student Health Services, or Mason's Title IX Coordinator (703-993-8730; cde@gmu.edu).

Fig. P4 from https://www.semanticscholar.org/paper/The-use-of-Xenopus-oocytes-in-drug-screening.-Kvist-Hansen/62b3d0bcd44ee866e4683cf2e02c70fb7aeb867b