NEUROBIOLOGY LABORATORY

MCB 160L

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Notice to Students:

Read Exercise 1 before coming to first class meeting!

COURSE ORGANIZATION

Introduction

This course has several objectives. One is to introduce you to a variety of techniques used by neurobiologists to gain an understanding of how nervous systems work. Another is to develop exercises that improve your understanding of key principles in neuroscience. A third is to give you some idea of the skills that must be developed to be able to do neurobiological research. A fourth is to give you some idea of what it is like to grapple with neurobiological problems and seek experimental evidence to test hypotheses of how things work. A fifth is to provide you with enough familiarity with some aspects of neurobiological research that you can engage in independent honors research in faculty laboratories. Lastly, we hope this course will help you decide whether you are suited for a career in neurobiological research.

Neurobiology is a very diverse field. The nervous system is studied by an incredible variety of techniques in electrophysiology, biochemistry, anatomy, cell biology, molecular biology, genetics, behavioral analysis, biophysics, psychophysics, theoretical modeling, etc. No one is conversant with all these techniques, even those who are experts at some of them. There is no time to even expose you to all of these techniques. Rather, this course will select a few examples, from electrophysiology, cell biology, psychophysics, computer modeling, molecular biology, genetics, and anatomy. Experiments will be done on invertebrates, simple vertebrates, and humans, and will cover molecular channel properties, neuronal cell properties, organ and system properties, and behavior. Some techniques, like neurochemistry and molecular neurobiology, have been omitted because the techniques are essentially identical to those used in other areas of biochemistry. They will be used, however, in the preparation of specimens for study, and their roles in the exercises will be explained.

Other areas, like ultrastructural anatomy and magnetic resonance imaging, have been omitted because the techniques are simply too difficult to even develop a feel for in a few weeks, or because the equipment is prohibitively expensive (individual instruments costing over $1,000,000). Hopefully, the experiments chosen will give some idea of the breadth of techniques used and problems studied in neurobiology. If not, the instructor is always open to suggestions for alternative exercises.

The exercises in this course are stylistically quite diverse. But few of them are easy. You will need to learn to use new, complex, and unfamiliar equipment in short order. You will be developing skills such as fine dissection that normally take months to perfect. In each exercise we try to suggest more experiments than can normally be completed in the time available. The idea is to assure that the brightest students, the hardest workers, and those with the most luck, are still challenged and never bored!

Each exercise in this manual comes from a different source. Some were adapted and expanded from published undergraduate neurobiology laboratory manuals. Others were simplified from graduate laboratory courses taught elsewhere in the U.S. and Europe. Some were developed from scratch right here at Berkeley. All undergo continuing evolution and (we hope) improvement. Student input has had important effects on the exercises in the past. If you have specific criticisms and explicit suggestions for improvement (e.g., if you can correct errors or propose clarifications), your input will be most valued and will be taken very seriously.

You will gain the most from this course if you read the laboratory manual pertaining to each week's work before coming to class. The Graduate Student Instructor leading your laboratory section tends to get very annoyed when asked questions that are answered clearly in the syllabus. However, s/he will gladly answer questions about things that are not clear in the manual. The key to getting quality help from the GSI is to show that you have done your homework. This applies even to the very first exercise. Therefore, we ask that you read Exercise 1 before the first class meeting.

This course is meant to accompany, or follow, a basic lecture course in neurobiology. Completion of such a course is not, however, absolutely necessary, as long as you have read some sort of general introduction to the basic concepts and language of neuroscience (see Supplementary Readings). A lower division course in biology is, however, absolutely essential. You will also find the normal lower division courses in physics, chemistry, and mathematics to be very useful. An introductory course in (bio)physical chemistry would also be valuable.

Reports

The grade in this course will be based on two types of laboratory reports and on periodic exams. For exercises 1, 5, 6, 7, 9, 10, and 12, you will hand in a short report. This report will normally be a 1- to 4-page form in which you fill in the blanks. You will be asked to answer questions about your experiments, and to recount or draw your results. This report may be due at the end of the exercise, or in some cases, it is to be submitted at the next class meeting (one-half week later). Note that the short report forms are not substitutes for this syllabus. They do not describe how to do the experiments, nor everythging that is to be done. Only the syllabus does this, and its directions must be followed unless you are explicitly told otherwise.

For exercises 2, 3, 4, 8, 11 and 13, you will hand in a long report. This is a free-form report in which you describe your experimental results and answer questions posed in the laboratory syllabus. Do not write a scientific paper; omit introductions, methods, and discussion. Just state what you did and what you saw, and include all your data (usually in the form of photographs or Xerox copies of your partner's photographs). Brevity, completeness, and evidence that you understood what you did are rewarded with high grades. This report is due one week after completing the laboratory exercise; bring it to class the following week.

The following regulations and instructions are adapted from the recommendations for course syllabi of the Associated Students of the University of California (ASUC):

> The student community at UC Berkeley has adopted the following Honor Code: "As a member of the UC Berkeley community, I act with honesty, integrity, and respect for others." The hope and expectation is that you will adhere to this code.

Collaboration and Independence: Reviewing lecture notes and experimental results and studying for exams can be enjoyable and enriching things to do with fellow students. This is recommended. However, final written laboratory reports are to be completed independently and materials submitted as homework should be the result of one's own independent work. You will of course collaborate with your lab partners in collecting, analyzing, and trying to understand the data. All members of a lab group will normally submit a copy of the same **results, data, photographs, etc.** in their reports. But the final report, whether long or short, is to be written individually by each student. The report represents your work, your effort, your understanding, your ability to present your results and discuss them intelligently, and your answers to the questions posed in the exercises in your own words. The section instructors are quite adept at identifying **plagiarism**, and no credit will be given to duplicate reports or sections with very similar wording. Evidence of plagiarism may also be reported to the Center for Student Conduct for disciplinary action.

Cheating: A good lifetime strategy is always to act in such a way that no one would ever imagine that you would even consider cheating. Anyone caught cheating on an exam in this course will receive a failing grade in the course and will also be reported to the University Center for Student Conduct. In order to guarantee that you are not suspected of cheating, please keep your eyes on your own materials and do not converse with others during exams.

Academic Integrity and Ethics: Cheating on exams and plagiarism are two common examples of dishonest, unethical behavior. Honesty and integrity are of great importance in all facets of life. They help to build a sense of self-confidence, and are key to building trust within relationships, whether personal or professional. There is no tolerance for dishonesty in the academic world, for it undermines what we are dedicated to doing – furthering knowledge and discovering the truth for the benefit of humanity.

Your experience as a student at UC Berkeley is hopefully fueled by passion for learning and replete with fulfilling activities. We appreciate that being a student can be stressful. There may be times when there is temptation to engage in some kind of cheating in order to improve a grade or otherwise advance your career. This could be as blatant as having someone else sit for you in an exam or in a class exercise, or submitting a written assignment that has been copied from another student. And it could be as subtle as glancing at a fellow student's exam when you are unsure of an answer to a question and are looking for some confirmation. One might do any of these things and potentially not get caught. However, if you cheat, no matter how much you may have learned in this class, you have failed to learn perhaps the most important lesson of all.

If you must miss an exercise or an exam due to a need to be out of town, you must make prior arrangements with the GSI to make up the exercise. If you miss an exercise due to illness or other unanticipated emergency, you must explain the problem to your GSI as soon as possible to arrange for completing the work. The GSI has the right to insist on written proof on official letterhead of your reason for absence. A report not handed in, or an exam missed without prior arrangement or legitimate proof of excuse, earns zero points.

Grades

Grades will be determined by total number of points earned as follows:

Exams:	2 X 50 = 100 points
Long Reports:	3 X 20 = 60 points
Crayfish, Oocyte, Neuro-Optics Reports:	3 X 25 = 75 points
Short Reports:	7 X 10 = 67 points
	Total = 305 points

Letters of Recommendtion

Some students may wish to have an instructor in this course write a letter of recommendation supporting applications to professional, graduate, or medical school, or for employment. All such letters are handled through the campus Letter Service. You need to register with that service, obtain a recommendation form (with or without ranking grid – your choice) and a waiver of your right to see this letter, and contact by email the GSI of your choice who you think knows you best. The GSI will write a first draft and send it with your form and signed waiver to a faculty instructor (again, you choose the faculty member who you think knows you best). In consultation with the GSI, the faculty instructor will revise the letter as necessary, and send it to the Letter Service. This procedure combines the knowledge of the GSI with the experience of the faculty to produce the most effective letter. You designate recipients to the Letter Service, who will send all letters. If asked by an institution to identify letter writers, you may list the faculty instructor overseeing your letter, but give the Letter Service email address, following instructions on https://career.berkeley.edu/Letter/FAQ-send.

Sexual Misconduct

Current University regulations now require that the following statement be included in course syllabi:

> **Safe, Supportive, and Inclusive Environment.** Whenever a faculty member, staff member, post-doc, or GSI is responsible for the supervision of a student, a personal relationship between them of a romantic or sexual nature, even if consensual, is against university policy. Any such relationship jeopardizes the integrity of the educational process.
>
> Although faculty and staff can act as excellent resources for students, you should be aware that they are required to report any violations of this campus policy. If you wish to have a confidential discussion on matters related to this policy, you may contact the Confidential Care Advocates on campus for support related to counseling or sensitive issues. Appointments can be made by calling (510) 642-1988.
>
> The classroom, lab, and work place should be safe and inclusive environments for everyone. The Office for the Prevention of Harassment and Discrimination (OPHD) is responsible for ensuring the University provides an environment for faculty, staff and students

that is free from discrimination and harassment on the basis of categories including race, color, national origin, age, sex, gender, gender identity, and sexual orientation. Questions or concerns? Call (510) 643-7985, email ask_ophd@berkeley.edu, or go to http://survivorsupport.berkeley.edu/.

Laboratory Section 1 (Spring, 2017) TuTh 12-4 PM Course Control No. 18858

Week	Date	Exercise	Description	Report	Instructor
1	Tu Jan 17	1	Use of electrophysiological equipment	↓	Zucker
	Th 19		Use of electrophysiological equipment	Short	Zucker
2	Tu 24	2	Nerve excitability (frog sciatic)	↓	Zucker
	Th 26		Nerve excitability (frog sciatic)	Long	Zucker
3	Tu 31	3	Voltage clamp computer simulations	↓	Zucker
	Th Feb 2		Voltage clamp computer simulations	Long	Zucker
4	Tu 7	4	Intracellular recording 1: Dissection and recording	↓	Zucker
	Th 9		2: Effect of potassium and seeing EJPs	↓	Zucker
5	Tu 15	4	3: Correlated nerve and muscle recordings	↓	Zucker
	Th 16		4: Patterned nerve stimulation, EJPs and IJPs	↓	Zucker
6	Tu 21	4	5: Neuromodulatory control	Long	Zucker
	Th 23	5	EEG & human evoked potentials	Short	Zucker
7	Tu 28	6a & 6b	Mechanoreceptors; Human sensory & motor systems **(& EVENING MIDTERM EXAM, 4056 VLSB, 7-9 PM)**	Short	Zucker
	Th Mar 2	7	Image Processing and Neuron Outgrowth	Short	Zucker
8	Tu 7	8a	Expression of transmitter-activated ion channels	↓	Kramer
	Th 9		Expression of transmitter-activated ion channels	↓	Kramer
9	Tu 14	8b	Expression of voltage-gated ion channels	↓	Kramer
	Th 16		Expression of voltage-gated ion channels	Long	Kramer
10	Tu 21	9	Neuron cytoskeleton: immunocytochemistry	↓	Larue
	Th 23		Neuron cytoskeleton: immunocytochemistry	Short	Larue
--	Tu 28	--	SPRING RECESS		
	Th 30		SPRING RECESS		
11	Tu Apr 4	10	Brain slice: long-term potentiation	↓	Zucker
	Th 6		Brain slice: epileptic focus	Short	Zucker
12	Tu 11	11	Optical Recording of Neural Activity	↓	Zucker
	Th 13		Optical Recording of Neural Activity	Long	Zucker
13	Tu 18	12	Mammalian neuroanatomy	Short	Larue
	Th 20	13	Neurogenetics 1: mutations & behavior	↓	Ball
14	Tu 25	13	Neurogenetics 2: RNAi and development	↓	Ball
	Th 27		Neurogenetics 2: RNAi and development	Long	Ball
(15)	W May 10		FINAL EXAM – Location TBD		Zucker
	7:00-10:00 PM		Exam Group 12		

N.B. Excercises are in a more-or-less logical order, with more advanced exercises always following simpler ones on which they depend methodologically. Availability of shared equipment also affects order.

Laboratory Section 2 (Spring, 2017) WF 12-4 PM Course Control. No. 18859

Week	Date	Exercise	Description	Report	Instructor
1	W Jan 18	1	Use of electrophysiological equipment	↓	Zucker
	F 20		Use of electrophysiological equipment	Short	Zucker
2	W 25	2	Nerve excitability (frog sciatic)	↓	Zucker
	F 27		Nerve excitability (frog sciatic)	Long	Zucker
3	W Feb 1	3	Voltage clamp computer simulations	↓	Zucker
	F 3		Voltage clamp computer simulations	Long	Zucker
4	W 8	4	Intracellular recording 1: Dissection and recording	↓	Zucker
	F 10		2: Effect of potassium and seeing EJPs	↓	Zucker
5	W 15	4	3: Correlated nerve and muscle recordings	↓	Zucker
	F 17		4: Patterned nerve stimulation, EJPs and IJPs	↓	Zucker
6	W 22	4	5: Neuromodulatory control	Long	Zucker
	F 24	5	EEG & human evoked potentials	Short	Zucker
7	W Mar 1	6a & 6b	Mechanoreceptors; Human sensory & motor systems **(& EVENING MIDTERM EXAM, 4056 VLSB, 7-9 PM)**	Short	Zucker
	F 3	7	Image Processing and Neuron Outgrowth	Short	Zucker
8	W 8	8a	Expression of transmitter-activated ion channels	↓	Kramer
	F 10		Expression of transmitter-activated ion channels	↓	Kramer
9	W 15	8b	Expression of voltage-gated ion channels	↓	Kramer
	F 17		Expression of voltage-gated ion channels	Long	Kramer
10	W 22	9	Neuron cytoskeleton: immunocytochemistry*		
	F 24		Neuron cytoskeleton: immunocytochemistry*		
--	W 29	--	SPRING RECESS	↓	Zucker
	F 31		SPRING RECESS	Short	Zucker
11	W Apr 5	10	Brain slice: long-term potentiation	↓	Larue
	F 7		Brain slice: epileptic focus	Short	Larue
12	W 12	11	Optical Recording of Neural Activity	↓	Zucker
	F 14		Optical Recording of Neural Activity	Long	Zucker
13	W 19	12	Mammalian neuroanatomy	Short	Larue
	F 21	13	Neurogenetics 1: mutations & behavior	↓	Garriga
14	W 26	13	Neurogenetics 2: RNAi and development	↓	Garriga
	F 28		Neurogenetics 2: RNAi and development	Long	Garriga
(15)	W May 10		FINAL EXAM – Location TBD		Zucker
	7:00-10:00 PM		Exam Group 12		

N.B. Excercises are in a more-or-less logical order, with more advanced exercises always following simpler ones on which they depend methodologically. Availability of shared equipment also affects order.

Lecture Schedule (Spring 2017)

Course Control No. 18857

Mondays, 3-4 PM, 101 LSA

Jan. 23	Nerve excitability (RZ)
Jan. 30	Voltage clamp simulations (RZ)
Feb. 6	Crayfish synapse: intracellular recording (RZ)
Feb. 13	EEG
Feb. 20	PRESIDENTS' DAY HOLIDAY
Feb. 27	Mechanoreceptors; human sensory & motor; image processing & neural outgrowth (RZ)
Mar. 6	Expressing transmitter receptors (RK)
Mar. 13	Expressing ion channels (RK)
Mar. 20	Neuron Cytoskeleton & Immunocytochemistry (DL)
Mar. 27	SPRING RECESS
Apr.3	Brain Slice (RZ)
Apr. 10	Neuro-Optics (RZ)
Apr. 17	Neurogenetics 1: mutations & behavior (RB)
Apr. 24	Neurogenetics 2: RNAi and development (RB)

Faculty: RZ: Robert Zucker; RK: Richard Kramer;
DL: David Larue; RB: Robin Ball

Supplementary Readings for MCB 160L

(on reserve in the Bioscience Library)

These selected readings have been taken from the following sources:

Animal Physiology by Roger Eckert and David Randall, W.H. Freeman & Co., S.F., 1978 (Pam 1)

Journal of Experimental Biology, Vol. 43, 1965 (Pam 7)

Fundamentals of Sensory Physiology by Robert F. Schmidt, Springer-Verlag, N.Y., 1978 (Pam 2, 4)

Textbook of Medical Physiology by Arthur C. Guyton, W.B. Saunders Co., Philadelphia, 1976 (Pam 2,3,4)

Introduction to Nervous Systems by Theodore H. Bullock, W.H. Freeman & Co., S.F., 1977 (Pam 6)

Frog Neurobiology, a Handbook. ed. by R Llinás and W. Precht, 1976 (Pam 4)

Unpublished, by Peter Getting (Pam 7)

Cold Spring Harbor Symposia on Quantitative Biology, Vol. 55, 1990 (Pam 8)

Nature Methods, Vol. 2, 2005 (Pam 9)

Differentiation, Vol. 72, 2004; Nature, Vol. 418, 2002 and Vol. 433, 2004 (Pam 10)

Cell, Vol. 131, 2007; Cell Calcium, Vol. 47, 2010 (Pam 11)

Biophys. J., Vol. 54, 1988 (Pam 12)

Exercise 2 Propagation of Nerve Impulses	Pamphlet 1
Exercise 3 Etiology of the Supernormal Period	Pamphlet 12
Exercise 4 A. Electrophysiological Recording (What They Normally Don't but Should Tell You) B. Reflex Control of Abdominal Flexor Muscles in the Crayfish	Pamphlet 7
Exercise 5 Nonspike Signaling: Electric Fields of Units and Assemblies	Pamphlet 6
Exercise 6 1. A. Physiology of Vision (selections) B. Autonomic Control of Accommodation and Pupillary Aperature C. Color Vision	Pamphlet 2
2. A. Physical Principles of Optics B. The Optics of the Eye C. The Ophthalmoscope	Pamphlet 3
3. A. Somato-Visceral Sensibility (selections) B. Physiology of Hearing (selections) C. Hearing Abnormalities D. Physiology of the Sense of Equilibrium E. Vestibular Mechanism for Stabilizing the Eyes and for Nystagmus F. Clinical tests for Integrity of Vestibular Function	

G. Physiology of Taste (selections) Pamphlet 4

4. Cutaneous Mechanoreceptors Pamphlet 5

(Pamphlets 2, 3, and 4 are for Exercise 6B; pamphlet 5 is for Exercise 6A)

Exercise 8

Molecular Studies of Voltage-Gated Potassium Channels Pamphlet 8

Exercise 10

Fluorescence Microscopy Pamphlet 9

Exercise 11

Calcium Signaling

SOCIC: The Store-Operated Calcium Influx Complex Pamphlet 11

Exercise 13

A. RNA Interference

B. High-Throughput RNAi in Caenorhabditis elegans

C. Unlocking the Potential of the Human Genome with RNA Interference Pamphlet 10

In addition to the selections from books and journals collected as pamphlets, the following books may be useful for the exercises indicated, and have been placed on reserve for this course in the Bioscience Library:

A very basic introduction to neuroscience that can be read in one evening

Neurophysiology: A Primer by C. F. Stevens. John Wiley & Sons. Chapters 1-9.

Exercise 1

Physical Techniques in Biological Research by W. L. Nastuk, Vol. V, Electro-physiological Methods Part A, Academic Press, 1964. See especially chapters 1, 2, 3, 5, 7, 8, & 9.

Exercise 2

Physiology and Biophysics by T. Ruch and H. D. Patton, Vol. 4, Excitable Tissues and Reflex Control of Muscle, W. B. Saunders Co., 20th ed., 1982. See especially chapter 4.

The Physiology of Excitable Cells by D. J. Aidley, Cambridge Univ. Press, 3rd ed., 1989. See especially chapter 4.

Exercise 3

Ionic Channels of Excitable Membranes by B. Hille, Sinauer Asso., 2nd Edn., 1992. See especially chapter 2.

Exercise 5

Human Brainwaves: the Psychological Significance of the Electroencephalogram by J. Empson, Stockton Press, 1986.

Exercise 7

Video Microscopy: The Fundamentals by S. Inoue and K. R. Spring, Plenum Press, 1997.