Bowdoin College is a small liberal arts college that offers a comprehensive Neuroscience major. The laboratory experience is an integral part of the major, and many students progress through three stages. A core course offers a survey of concepts and techniques. Four upper-level courses function to give students more intensive laboratory research experience in neurophysiology, molecular neurobiology, social behavior, and learning and memory. Finally, many majors choose to work in the individual research labs of the Neuroscience faculty. We, as laboratory instructors, are vital to the process, and are actively involved in all aspects of the lab-based courses. We provide student instruction in state of the art techniques in neuroscience research. By sharing laboratory teaching responsibilities with course professors, we help to prepare students for careers in laboratory neuroscience and also support and facilitate faculty research programs.

Key words: laboratory instruction; neurophysiology; neuroanatomy; social behavior; learning and memory; molecular neurobiology; liberal arts education

INTRODUCTION

Bowdoin is a small liberal arts college that offers a comprehensive neuroscience major. There are currently four professors and two laboratory instructors dedicated to the Neuroscience program. The authors of this paper serve as laboratory instructors in five different neuroscience courses, all of which have an integral laboratory component. In this paper, we offer our perspective as neuroscience instructors, describing our role in preparing students for participation in faculty research programs, both at Bowdoin and in post-graduate careers.

At Bowdoin, laboratory instructors have served an essential role in laboratory teaching for more than twenty-five years. By sharing many of the laboratory teaching responsibilities, laboratory instructors enable professors to maintain an active research program despite the extensive teaching responsibilities at a liberal arts college. Laboratory instructors also facilitate the integration of undergraduates into faculty research laboratories, allowing research faculty to spend more time mentoring the undergraduates in their labs. Most neuroscience courses have a very intense laboratory component, and laboratory instructors are instrumental. Although the role of the laboratory instructor varies from course to course, we typically develop laboratory exercises and teach the labs in collaboration with the course professor. We are responsible for performing trial runs of the exercises, troubleshooting and fine-tuning the techniques, writing many of the laboratory protocols, preparation of other course materials such as dissection videos, and grading student laboratory assignments that often take the form of scientific papers. Laboratory instructors at Bowdoin are hired as much for their teaching experience as their area of expertise. One of us, Stephen Hauptman, came to Bowdoin with advanced degrees in Human Biology and Vertebrate Zoology. Nancy Curtis came to Bowdoin with a Master of Science degree in functional morphology, and a broad background in anatomy and physiology. Both of us learned many of the technical procedures we now teach under the tutelage of the neuroscience professors at Bowdoin.

Our core course is named, simply, Neurobiology, and it serves as a laboratory foundation for four research-intensive upper-level courses. The student lab exercises in Neurobiology cover a range of neuroscience research areas. Each of the four neuroscience professors teaches an upper-level course directly relevant to their research expertise. Dr. Patsy Dickinson teaches Neuropsychology, Dr. Hadley Horch teaches Molecular Neurobiology. Dr. Seth Ramus teaches Laboratory in Behavioral Neuroscience: Learning and Memory, and Dr. Richmond Thompson offers Laboratory in Behavioral Neuroscience: Social Behavior. Two professors (Dickinson and Horch) have a joint appointment in Biology, and two (Thompson and Ramus) have a joint appointment in Psychology.

Neuroscience majors are required to take at least three of these upper-level courses, guaranteeing a broad research background for all majors. These courses all include laboratory projects that take up a substantial part, or even the entire duration, of the semester. The close integration with faculty research expertise promotes student research that is current and very often uses novel experimental techniques. This focus on student-generated research begins on a smaller scale in Neurobiology, the core course, and many majors eventually expand on these skills with Independent Study or Honors projects in the research labs of the neuroscience faculty. Through this sequence of courses, students are systematically introduced to both the demands and rewards of scientific research.

THE COURSES

Neurobiology

The challenge of a lab sequence that covers such a wide range of neuroscience subdisciplines is to engage the students so that they see the unifying threads of neuroscience and to ensure a cohesive learning experience as they move from one topic to another.
Neuroscience has many areas of study, is interdisciplinary, and attracts students with a wide variety of backgrounds and interests. All students enter the course sequence with a background that includes Introductory Biology, but apart from this, some students have taken more psychology courses, while others have concentrated on biology.

Neurobiology is a fairly new course, and initially the lab sequence began with our introduction to the techniques and concepts of electrophysiology, based on a sequence of three labs from the Crawdad manual (Wyttenbach et al., 2002). We introduced the students to electricity and the model neuron one week, following up on this with the crayfish Nerve 3 and crayfish stretch receptors (MRO) recordings in later lab periods. We took a fairly discrete approach to the three exercises. For the model axon lab, we invited an instructor from the physics department to introduce basic electrical concepts before the class followed the Crawdad exercise. For the two recordings, students designed short experiments and wrote them up as scientific format reports. These are good, tested labs. The students ran what we saw as successful experiments, and many of the scientific format reports we asked them to write were quite good.

We discovered from course evaluations that, for many students, this was a less successful sequence than we had hoped. Some students didn’t understand how the introduction to electricity was relevant to the animal exercises that followed, or the biological significance of their recorded traces. Others were at a loss as to what experiments were appropriate, and how to go about writing a report based on data they didn’t completely understand. What seemed so basic to us, that in many ways neurophysiology is electricity, was a foreign, novel concept to many students, and they were then doing their first electrophysiological recordings and experiments with no real grasp of the concepts so basic to extracellular recording, or the skills necessary to put together a report.

In response to the comments, we revamped this introductory electrophysiology sequence. We made it more unified and focused, and more directly coordinated with the corresponding class lectures. We developed a three-week sequence that took much less for granted, and more explicitly made the connections that many students weren’t seeing. We still introduce students to basic electrical principles, but we do so while constructing on the blackboard the model neuron, simultaneously explaining the biological significance of the resistors chosen to make the neuron. Our power source example is the battery they will use in the model neuron exercise to simulate an action potential.

To introduce the principles of data acquisition, and to illustrate just how simple the concept of extracellular recording is, we use weakly electric fish, as suggested in the first Crawdad lab. With just a couple of copper wires as electrodes, we demonstrate what happens to the recording as the fish moves around the aquarium. Students are able to see the biological nature of the signal itself, and also observe the changing amplitude of the signal as the fish moves closer and further from the electrodes. We also use the electric fish to demonstrate the importance of sampling speed in accurately recording the fastest action potentials. We use a sample trace from the lab the students will be doing the next week, the Nerve 3 recording, to demonstrate on an actual biological recording the significance of the different sizes of extracellular action potentials and what it can reveal about neuron diameter. For their first electrophysiological recording, the students clip a hind leg off a cockroach and, using electrodes that consist of insect pins soldered to a cable, they pierce the femur in two places along the femoral nerve, and use a glass probe to stimulate the tibial hairs to generate and record action potentials in the nerve. A variant on this experiment can be found in Ramos et al. (2007).

The students are not asked to analyze these recordings in any way; the goal is to make them comfortable with extracellular recordings, and demonstrate to them that neurons don’t have to be connected to a whole animal, or even immersed in an artificial saline, to be functional. The isolated cockroach leg recording, in particular, makes a dramatic impression. Many students are astonished to see biological, electrical activity from a seemingly “dead,” detached part of an insect.

We still follow this first week of lab with a recording from the crayfish Nerve 3, but use the recording to more specifically look at the anatomy of the nerve. The students simply record spontaneous activity from this nerve using a suction electrode. This is a much less intimidating introduction to more sophisticated extracellular techniques. In the same prep, using a process developed by Dr. Hadley Horch, they prepare the nerve for a fluorescent fill. We do this fill in situ, cutting out a small piece of the superficial flexor with a length of the nerve attached to it. The students prepare a bed of petroleum jelly, move the nerve across it, and build a petroleum jelly well around a small section of the intact nerve. A drop of deionized water is dropped into the well, and the nerve is cut. The water is replaced with a conjugated fluorescent dye. After allowing the nerve to fill overnight, the nerve is removed along with a stretch of the ventral nerve cord with the ganglia both anterior and posterior to the nerve. It is fixed for several days, and mounted on slides the next week. They look at the slides through fluorescent microscopes and each group also looks at their slides through the confocal microscope, recording images from both microscopes.

The physiological recordings are used to determine the number and relative diameters of the axons in the nerve by sorting the classes of action potential amplitudes, and the microscope images are used to visualize the axons, and correlate their diameters with the recorded action potential amplitudes (Figure 1). A combined physiology/anatomy lab is used to teach the principles of electrophysiology, introduce microdissection, microscopy technique, and demonstrate the use of fluorescent dyes to visualize neurons.

Instead of asking them to then write a scientific format report, the students are led through a worksheet that asks the questions normally addressed in a report. We show them what needs to be reported about this experiment, giving more direction than we had in past semesters.
Later in the semester we take a behavior/anatomy approach to explore the effects of locomotor mutations at neuromuscular junctions among *Drosophila* with Shaker/eag mutations. By tracing 3rd instar crawling patterns on agar-coated plates, students compare behavioral crawling differences among the various mutants (Wang et al., 2002). Using fluorescent dyes, they make slides and visualize the differences in the morphology of the neuromuscular junctions that correspond to the behavioral differences (Budnik et al., 1990). This is written up as a scientific-format report bringing the two different ways of looking at the effects of the mutations on the morphology of the neuromuscular junctions and the effects on crawling behavior into one cohesive analysis (Figure 2). Drs. Pat Rivlin and Ron Hoy provided valuable advice and assistance in the development of this lab exercise.

Figure 1. A) Extracellular recording of the action potentials from the six different neurons in the crayfish Nerve 3. B) Confocal microscopy image of the Crayfish Nerve 3 showing axons of all six neurons.

Figure 2. A) A confocal microscopy image of the wild type *Drosophila* neuromuscular junction at the Muscle 6/7 stained by AlexaFluor 594. B) Crawling patterns of Wild Type (top) and eag 3rd Instar larvae. C) Neuromuscular junction at Muscle 6/7 of an eag *Drosophila* mutant.

In our sensory unit, we use the Psy-Cog CD-ROM (Wyttenbach, 2006) to introduce the students to concepts of visual perception, and simultaneously introduce the use of statistics and scientific graphing. The students choose from the many visual illusions on this CD-ROM and design an experiment. They recruit friends and classmates to be their subjects, creating a sample size large enough for parametric statistics. This experiment is also written up as a scientific format report.

Other labs include a basic sheep-brain anatomy lab, and a cricket aggression behavior lab. We like to conclude the semester with a lab that is still in the beta stage of development. One of the more ambitious labs that we are still developing uses DiOlistics to stain individual neurons in fixed mouse brain slices (Morales et al., 2006). The students are introduced to a very recently developed technique (Gan et al., 2000), and get practice using brain atlases to identify the portion of the brain to which the slices belong. They hypothesize the morphology of neurons in various regions of the brain and look at slides in which some individual neurons express GFP (Feng et al., 2000) and others are stained with the DiOlistics dye (Figure 3).

Figure 3. A) A cortical neuron stained through the DiOlistics process. B) Mouse cortical pyramidal cells expressing GFP.

UPPER LEVEL COURSES

The core-level course, Neurobiology, utilizes a variety of approaches to accommodate a range of student backgrounds and prepare students for the very different focus of the upper level courses. In the laboratory component of each of these courses, the goal is to train students in the techniques appropriate to the specific focus of the course while engaging them in rigorous research that reinforces the course concepts. The format is versatile enough to include different pedagogical approaches to accomplishing this common goal.

Neurophysiology

In Neurophysiology, the students spend half of the semester learning basic neurophysiology concepts and techniques. A week is devoted to mastering the instrumentation, learning to fill microelectrodes and manipulate them into a dish of saline. Stimulator pulses serve as the signal the students record through the data acquisition software. Once students are confident they can complete the process on their own, we ask them to leave the room for a few moments while we systematically undo everything they did to get to that point, changing all the switches, settings, and dial positions on the DC amplifier, the oscilloscope, the stimulator, and the data acquisition software. They then start from scratch, returning the instrumentation to the settings necessary to make their first intracellular recordings.

A basic resting membrane potential lab uses a frog sartorius muscle. This introduces the students to
intracellular recordings on easily penetrated, easily visualized muscle fibers, while learning the basis of the resting membrane potential by making the recordings in various concentrations of external potassium. We have added a new aspect to this lab, working with students in a mathematical modeling course to model the data based on the Goldman-Hodgkin-Katz equation.

The students are introduced to intracellular recordings from neuron cell bodies using the central ganglia of pond snails (Hauptman et al., 2006). The use of dual head dissecting scopes, precision micromanipulators, and a dissection video produced by the laboratory instructor (http://www.bowdoin.edu/faculty/s/shauptma/videos.shtml) have dramatically increased the student recording success rate. The first week is devoted to practicing penetrating cells and looking at basic properties of action potentials, as virtually every cell shows spontaneous activity. During the next lab period we use the spontaneous compound post-synaptic potentials in the bursting cells of the buccal ganglia to experimentally differentiate inhibitory and excitatory psps, determine reversal potentials, and explore the underlying pattern generator driving the bursting cells.

The second half of the semester is devoted to individual group projects. Many of the studies involve modulation of simple neural networks, which is the primary research interest of the course professor, Dr. Patsy Dickinson, but the research projects range much wider than this. We offer a limited number of organisms and systems from which the students can choose. The most commonly used systems include the pond snails *Lymnaea* and *Helisoma*, *Drosophila* larvae, crickets and crayfish. We try to add to the available systems with occasional exploratory labs introducing a new system. Visits from Drs. Bruce Johnson and Stefan Pulver added *Drosophila* larvae to the choices. We hope to offer a similar introductory lab to the use of *Manduca* in the coming year. Some of the projects are quite ambitious, and some serve as pilot studies for Honors projects.

Some examples of studies that have been done include attempts to establish physiological evidence for the presence of NMDA receptors in the buccal ganglia neurons of pond snails or at the *Drosophila* neuromuscular junction. Students have looked at evoked epileptiform activity in *Helix* and *Helisoma*, and the neural basis of *Lymnaea* learning.

**Molecular Neurobiology**

The model system used in Molecular Neurobiology is the cricket *Gryllus bimaculatus*. The focus is the cellular and molecular level of biological organization. In accordance with the primary research of the course professor, Dr. Hadley Horch, the course explores and tries to define the mechanisms of compensatory synaptogenesis and neuronal plasticity in the central nervous system using the auditory system of *G. bimaculatus*. Each semester, the students make morphological comparisons between control animals and crickets that are unilaterally chronically deprived of auditory sensory input. Since cricket tympanic membranes are located on both front legs, the removal of the right front leg during early larval instars followed by weekly removal (by the laboratory instructor) of any regenerated tissue produces chronic unilateral sensory deprivation. The students use microdissection techniques to backfill interneuron AN-2 in the prothoracic ganglion of the cricket with biocytin conjugated to fluorescent dyes. The neuron is visualized using a confocal microscope. Morphological comparisons are made between the two conditions (Figure 4).

![Figure 4. Retrograde neural tracing done in the classroom. Dotted line with white arrow marks the midline of the ganglion.](image-url)

In each semester, a different potential molecular mechanism that may be driving the observed neuronal plasticity is studied. Methods of inquiry include degenerate primer design, polymerase chain reaction (PCR), cloning, sequencing, cryostat sectioning, and *in situ* hybridization. One approach has been to start with differential display candidates (provided by the Horch research lab) that show up-regulation of mRNA species in the sensory deprived treatment groups compared to control groups. Essentially the students start out with an unknown candidate at the beginning of the semester, clone the candidate, then sequence the clones in an attempt to identify the candidate by using bioinformatics (Altschul et al., 1997). This approach identifies known molecular species (matching published sequences) and potential novel genes. Cloned sequences are used to make digoxigenin labeled RNA probes for *in situ* hybridization. All students use a cryostat to prepare frozen sections for the *in situ* run; the laboratory instructor prepares the probes and all the solutions required.

Students carry out the *in situ* (three-day protocol) procedure and then collect micrographs of the results and use this information combined with cloning results to construct a molecular-based model to explain compensatory growth and synaptogenesis in the cricket auditory system. Students are required to keep a detailed laboratory notebook that is graded twice a semester. Students write and revise a laboratory report to present and support the model they have proposed. Results from the molecular investigation and neuroanatomy work are
Laboratory in Behavioral Neuroscience: Social Behavior

The goldfish *Carrasius auratus* is the model system used in the Social Behavior laboratory course. The course focus is at the neuronal circuitry level of biological organization. The professor of the course, Dr. Rick Thompson, studies the molecular mechanisms involved in social approach behavior; what are the mechanisms that influence an organism to be in close proximity to a conspecific? This is the focus of the laboratory research in the course, and the format of this laboratory introduces students to the current interests and methodology used in Dr. Thompson’s research lab. Three to five modules are carried out each semester. Students develop an experimental design for each experiment they will carry out, although the course professor subsequently determines the specific experimental details. The behavioral testing paradigm (Thompson et al., 2004) is consistently used year to year with minor modifications, while the techniques used each semester vary. Procedures used for inquiry have been: primer design, polymerase chain reaction (PCR), isolation of total RNA, cDNA synthesis, cloning, brain removal and sectioning, in situ hybridization, and immunohistochemistry. RNAi silencing of the vasotocin gene by direct injection into brain ventricles has been performed, as has retrograde neural tracing of the vagal nerve from the heart to the brain and within the brain itself. There is some overlap between the techniques used in the Molecular Neurobiology and Social Behavior courses. The use of polymerize chain reaction (PCR) has been very successful in both courses. Through the use of PCR, we have found novel pieces of nucleic acids that match with innexins and Semaphorin 2a in crickets and a vasotocin receptor, and c-Fos gene in goldfish. Every new piece of nucleic acid identified has gone back into the faculty research program for further study.

Laboratory reports are written for each module completed. Students are also required to keep a detailed laboratory notebook that is graded twice during the semester. Students in Honors programs or undertaking independent projects continue successful experiments piloted in the teaching laboratory. One Honors student found a novel mRNA sequence for a vasotocin receptor in *C. auratus*, a research project started in the course.

Laboratory in Behavioral Neuroscience: Learning and Memory

The laboratory course in Learning and Memory uses the rat (Long-Evans) as the model system. The course focus is at the systems level of biological organization. Dr. Seth Ramus’s research addresses episodic memory and the roles of cortical structures and hippocampus for recent and remote memories. The entire semester is dedicated to running a single class experiment, collecting and interpreting the data, and writing up the project in Journal of Neuroscience format. The students are given a novel behavioral task for experimentation, and lead discussions of primary journal articles germane to the experiment on a regular basis throughout the semester.

In any given semester, the particular behavioral test is generated by Dr. Ramus based on his current research interests. This format is very versatile, allowing a broad range of behavioral testing techniques to be introduced while consistently providing instruction in fundamental techniques. The 2007 class conducted a water maze task that is being written up by students for publication.

In addition to running the behavioral task, the students perform these techniques each semester: classic ablation surgery via bilateral electrolytic lesion of the fornix (a proxy surgery for hippocampal ablation), trans-cardiac perfusion, tissue sectioning (frozen sections), staining tissue, routine light-level histology, morphological characterization of lesions across all experimental animals, and parametric data analysis. Additionally, students are trained in small mammal handling and maintaining records for research animals.

The course has also been taught in collaboration with other institutions (Yates et al., 2006). This class requires a substantial time commitment outside of scheduled labs.

CONCLUSION

Although the core course, Neurobiology, is not a requirement for enrollment in the upper-level courses, students choosing to take Neurobiology receive laboratory experience more specific to the discipline in addition to the basic biological lab techniques they learn in Introductory Biology. Students receive a very different lab experience in each of the upper-level courses. In Neuropsychology, students have a great deal of latitude in choosing a research project after completing a sequence of fairly traditional lab exercises designed to provide a technical and conceptual foundation. In Molecular Neurobiology, the anatomical documentation of the regenerative process is repeated each semester while the studied molecular mechanism that may drive the process changes from semester to semester. In the Social Behavior laboratory course, the behavioral paradigm addressing social approach behavior remains consistent while the molecular and histochemistry techniques vary. In the Learning and Memory lab course, a novel behavioral task is introduced each semester and is accompanied by consistent teaching of a suite of skills appropriate to the discipline.

Despite the differences, there are clearly similarities in approach among the upper-level courses. Each of the lab sequences is designed to expose students to the challenges of real science, training them in the appropriate techniques and engaging them in research projects that demonstrate how experimentation illustrates and expands scientific knowledge. We believe the students leave these courses with a better understanding of the course concepts and the realities of scientific research. Students often follow up on research initiated in all four of these courses as Honors students in the faculty research labs. The four courses function to offer students a clear idea of the focus and philosophy of the research experience they can expect in the lab of each faculty member. At the same time, faculty has a chance to screen potential Honors students in the teaching laboratory.
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