ARTICLE IFEL TOUR: A Description of the Introduction to FUN Electrophysiology Labs Workshop at Bowdoin College, July 27-30, and the Resultant Faculty Learning Community

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The workshop "Introduction to FUN Electrophysiology Labs" was organized by Patsy Dickinson (Bowdoin College), Steve Hauptman (Bowdoin College), Bruce Johnson (Cornell University), and Carol Ann Paul (Wellesley College). It took place July 27-30 2006 at Bowdoin College. There were fifteen participants, most of whom were junior faculty at college and universities around the country. This article describes the workshop content,

INTRODUCTION

In July 2005, Project Kaleidoscope (PKAL; www.pkal.org) and the Faculty for Undergraduate Neuroscience (FUN; www.funfaculty.org) held a workshop on undergraduate neuroscience education. One of the meeting's highlights was workshops held by Patsy Dickinson (Bowdoin College), Steve Hauptman (Bowdoin College), Bruce Johnson (Cornell University), and Carol Ann Paul participants (Wellesley College) to introduce to electrophysiology labs. Bruce introduced his house fly preparation in a workshop entitled "Maggot Neurobiology: A Neurophysiological Introduction to Drosophila as a Model System in Neuroscience." Steve and Carol Ann presented the Lymnaea preparation in the workshop "What Makes a Neuron: Intracellular Properties of Lymnaea." Both workshops were well received, but they were primarily demonstrations. When the leaders of these workshops debriefed after the PKAL meeting, they were impressed by the number of participants who came up afterwards with the question: Where can I learn to do physiology? Most people have heard of the Woods Hole summer workshops, which are intensive in nature and designed to teach neurophysiology in depth. However, the Woods Hole workshops are weeks to month-long workshops, requiring more time and effort than many faculty can afford. In addition, they are expensive and not geared towards teaching faculty.

As is the case following all PKAL workshops, Patsy, Steve, Bruce and Carol Ann felt the excitement to continue the energy of the workshop into their everyday lives. They came up with the idea of running a short (weekend) workshop to let instructors learn enough so that they could realistically return to their home institution and set up and run some physiology labs.

The workshop designed by this quartet consisted of

the incorporation of lab exercises at home institutions, and the faculty learning community that has resulted from the workshop.

Key words: electrophysiology, education, faculty learning community, faculty for undergraduate neuroscience

several experimental sessions in which the attendees practiced electrophysiological techniques, and concluded with a session on where to get equipment. The techniques covered included building and using an electronic model of the passive properties of an axon membrane, constructing a suction electrode, using equipment with data analysis software, and recording from several preparations (extracellular spontaneous motor activity in crayfish tail posture muscles, stimulus intensity coding in a stretch receptor, intracellular resting membrane potentials in crayfish, synaptic potentials at the crayfish neuromuscular junction (NMJ), electrical properties of snail neurons, and synaptic properties and bursting neurons in snail buccal ganglia). The final session detailed equipment needs and sources and supply costs.

In addition to these techniques, we were shown by example how to troubleshoot, and how to lead students through the process of a systematic examination of the setup and data to isolate and resolve technical problems.

Perhaps most importantly, our experience in the IFEL workshop formed a new kind of Faculty Learning Community. Research tends to be a collaborative enterprise, but teaching tends to be a solitary endeavor (Cox, 2004). To strengthen and improve the endeavor, teaching also needs to build similar collaborative networks. The Faculty for Undergraduate Neuroscience can help by providing more workshops that can provide instruction and then continue the faculty development by creating a network of professionals who continue to interact and support each other.

Sponsors of the IFEL (Introduction to FUN Electrophysiological Labs) workshop held at Bowdoin College July 27-30 2006 included Bowdoin College, NSF DUE-0231019, Faculty for Undergraduate Neuroscience, Edvotek (Bethesda, MD), and ADInstruments (Colorado

Springs, CO). As a consequence, registration fees for the meeting were a modest \$100, with \$250 for room and board in Bowdoin College residence halls.

DESCRIPTION OF THE TECHNIQUES COVERED IN THE WORKSHOP

Electronic model of the passive properties of an axon membrane. In the first section of the IFEL workshop. participants explored the passive electrical properties of biological membranes. This exercise is part of Lab 1 in the Crawdad exercises (Wyttenbach et al., 1999). Participants were shown how to construct an electrical model of half of membrane). an axon (one to simulate intracellular/extracellular recordings, and to observe the effects of capacitance on the time constant of a voltage pulse. This model axon consisted of a chain of resistors representing the resistance across the membrane (R_m) arranged parallel to each other. In addition, resistors representing the resistance along the neuronal membrane in the extracellular solution (R_o) and the intracellular fluid (R_i) were placed perpendicularly to the membrane resistance (Fig. 1). A battery was used to simulate the electrical potential traveling down the axon. In both intracellular and extracellular recording configurations (i.e., recording across R_m or across R_o), participants were asked to plot the voltage with respect to the position of the battery, compare between the two recording configurations, and calculate space constants for the model membrane.

In addition to using a model axon, a resistor-capacitor (RC) circuit was used to demonstrate the effects of capacitance on the electrical properties of a biological membrane. In this exercise, a stimulator was used to produce a voltage pulse across the RC circuit, and the input and corresponding output of the circuit were compared. The slowed rise time of the voltage was obvious. IFEL'ers were asked to calculate the time constant and to hypothesize how changing the time constant affects the temporal spread of voltage changes.

If the resistance ladders are already made, this exercise can be accomplished in 30-45 minutes, making it a good exercise to combine with an introduction to the equipment.

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Figure 1. Circuit representation of the artificial axon, as described above. Rm represents resistance across the membrane, Ri represents internal resistance, and Ro represents resistance outside the membrane.

Preparation of a suction electrode. Workshop participants were introduced to the fabrication of a simple extracellular suction electrode made from a plastic 10 ml pipette, conductor cable, a microelectrode holder, a 3-way luer stopcock, a syringe, and some tubing, used here for extracellular recordings of spontaneous motor activity in crayfish tail posture muscles and stimulus intensity coding

in a stretch receptor (see below). Suction electrodes of similar construction (to varying degrees) have been described elsewhere in the literature (Easton, 1993; Wyttenbach et al., 1999, Land et al., 2001; Yoshida, 2001), and have been used in undergraduate laboratories for recording from a variety of vertebrate and invertebrate nerve and muscle preparations. These previous descriptions relied on tips made of plastic gel-loading pipettes. In contrast, the method learned at the workshop resulted in an electrode holder that accommodates pulled microelectrode glass. The primary advantage of this difference is that the glass tip can be easily broken off, then fire-polished over an open flame, to enable a variablesized opening of user preference for diverse applications.

Use of equipment and data analysis software. The workshop was a hands-on experience for intracellular and various recording using extracellular invertebrate preparations including crayfish and snails. There were six rigs for pairs of faculty to engage in various recording exercises. All rigs included an anti-vibration table to dampen vibration. several micromanipulators for electrodes, a dissecting scope, and A-M Systems electronics including a Neuroprobe DC Amplifier Model 1600 for intracellular recording, a Differential AC Model 1700 amplifier for extracellular recording, and an Isolated Pulse Stimulator Model 2100, used to set duration, amplitude. and frequency of stimulus pulses. Oscilloscopes were also used but sparingly since data was fed into a PowerLab 2/20 (AD Instruments, Inc) unit which converted the analog signal to digital and allowed acquisitions by an iMac G5 computer. This allowed for real time observation of electrical activity and permanent storage of the data. General equipment circuits for each of the Crawdad labs are available in the Crawdad manual (Wyttenbach et al., 1999).

The PowerLab units use a software program called Chart which displays data like a chart recorder. The



Figure 2. IFEL participants familiarizing themselves with the equipment (picture by CA Paul).

PowerLab software acquires, stores, and analyzes data on both Macintosh and Windows platforms. Sampling rates of up to 10 K/s are possible. Other helpful features include the ability to make comments, a handy measurement tool, zoom screen, and data pads for rapid calculations. Spike Histogram is an additional extension program that quickly allows the experimenter to set the noise level, eliminate it from the analysis, and plot a histogram of responses binned according to amplitude size. Pairs of faculty plotted histograms for extracellular recordings from responses of nerve 3 (n3) in the crayfish.

We did not have this opportunity, but this exercise can be combined with measurements of the electric organ discharge (EOD) from an electric fish. The fish is used as a biological function generator to help students learn how to manipulate the electronic equipment and data acquisition and analysis systems.

Extracellular recording of spontaneous motor activity in crayfish tail posture muscles. This exercise is Lab 2 in the Crawdad program. Crayfish (*Procambarus clarkii*) are great to use because they are commercially available. They are easy to maintain in the lab at room temperature in an aerated freshwater aquarium. As invertebrates, they are not currently monitored by IACUCs (Institutional Animal Care and Use Committees) or other animal monitoring organizations. Most importantly, these animals have easily accessible robust muscles and nerves in their tails, and their NMJ integrates excitatory and inhibitory information, unlike vertebrate NMJs, which are excitatory only.

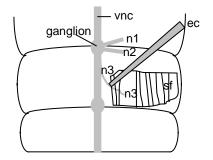


Figure 3. Drawing of tail segment with cuticle removed showing an extracellular recording electrode (ec) recording from a portion of n3. Also shown is the superficial flexor (sf) innervated by n3, the segment ganglion, nerves n1 and n2, and the ventral nerve cord (vnc).

To access the muscle and nerves of the crayfish for recording, the crayfish were anaesthetized on ice for several minutes. When animals became inactive, their tails were removed from their bodies and pinned ventral side up into a dissecting dish. The cuticle was removed to expose the ventral nerve cord and superficial flexor (sf) muscle of a tail segment. Ganglia associated with each sternite give rise to three motor neurons: the large n1 and n2, and the smaller n3, which is a pure motor nerve that fires tonically for tail postural control (Fig. 3). Neural activity was measured by pulling a portion of n3 into a workshop-built suction electrode connected to an AC amplifier. The

amplifier output was sent to a computer and to an audio monitor, allowing participants to both see and hear spontaneously firing action potentials. Using the Spike Histogram program enabled users to distinguish among neurons with different diameters, and thus, different extracellular action potential amplitudes and conduction rates. Further explorations, such as examining reflex activity, are detailed in Crawdad (Wyttenbach et al., 1999). Explorations of frog and earthworm nerve and muscle preparations (to investigate the role of myelin and other features using extracellular techniques) are available at www.adinstruments.com.

Stimulus intensity coding in a stretch receptor. This exercise (Crawdad Lab 10) used the same preparation as above, with the addition of a thread wrapped around the tail and attached to a micromanipulator. This time, however, the larger n2 nerve, which carries sensory information from muscle receptor organs (MROs) to the CNS, was the subject of our recordings. Pulling the thread, by moving the micromanipulator, caused the tail to curl and the MROs to fire. The audio for this exercise is especially rewarding because the stretch receptors provide robust firing. Excellent diagrams of the dissection, stimulus, and recording setup are available in Crawdad (Wyttenbach et al., 1999).

This combination of labs (n3 and stretch receptor) was a favorite exercise for many investigators at the IFEL workshop. The dissection is relatively simple and quick and the results are robust and exciting. Several different concepts are demonstrable with this preparation, including the correlation between stretch progress and cell firing, the response of stretch receptors to different rates of tail curling (MRO1 fires in response to slow or fast tail movements, while MRO₂ only fires after large rapid movements; Wyttenbach et al., 1999), and the adaptation of the response with continued stimulation. The nerves used for recording are easy to find along the inside surface of the tail shell and are accessible for attaching the suction electrode. The audio monitor is an essential part of this exercise; the stretch receptors provide great bursts of cell firing that are really best experienced by hearing the output.

Intracellular recordings of resting potentials in crayfish. This exercise (Crawdad Lab 4) uses the crayfish tail prep described above, except that the recording is with an intracellular glass electrode that penetrates a muscle fiber. The electrode output is connected to a DC amplifier hooked up to an oscilloscope and voltmeter. This arrangement makes possible the analysis of the effects of changing ion concentration, altering activity of the Na⁺/K⁺ pump, or adding pharmacological agents on the membrane potential. Experiments can be designed to reinforce the Nernst and Goldman equations, as well as the concept of the driving force of an ion.

This lab was more challenging than the extracellular recordings above. To ensure success, it is essential to optimize lighting and micromanipulator positioning before proceeding.

Synaptic potentials at crayfish NMJ. This exercise is Lab 8 in Crawdad. It involves simultaneously recording extracellularly from an intact n3 while also recording EPSPs intracellularly from the SF muscle. Note that the intracellular electrode must be near a synapse and not too deep to record EPSPs. This lab, especially, required careful attention to setup and lighting to achieve successful recordings.

Electrical properties of snail neurons. The next two exercises relied heavily on the Bowdoin neurobiology lab manual prepared by Patsy Dickinson and Steve Hauptman. While the manual is not currently available to the public, a manuscript is in preparation, and some of the anatomical background and other pertinent information including circuits and recordings are available in the literature (Benjamin et al., 1979; Safonova and Kiss, 1979; Winlow et al., 1982; Paul et al., 2006), or on websites (Eliott, 2003, http://biolpc22.york.ac.uk/snails/brains/bg.html; Murphy, http://tigger.uic.edu/~dmurphy/research/index.html). After anaesthetizing the snail in Listerine, the animal was cut out of its shell and pinned down onto a dissecting dish filled with dark sylgard (Fig. 4).



Figure 4. IFEL participants working on snail brain dissections (picture by CA Paul).

The mantle was folded back and cut through to the mouth so that the sex organs could be removed and the buccal mass pulled forward. The entire brain was removed, retaining as much of the long connectives for pinning as possible, and pinned into a clear sylgard dish with 0.1 minuten pins (Fig. 5A). After a brief 0.5% pronase wash and rinse with saline, the preparation was ready for recording using a glass electrode with resistance 10-40 $M\Omega$ filled with 0.6 M K₂SO₄. As in the previous exercise, this lab required extra care in the placement of the many pins, micromanipulators, electrodes, and lighting. We found that monitoring the pronase, angling the pins to ease approach by the electrode, and lighting from the side were essential. Most workshop participants had to use several snails in order to produce a viable preparation in good enough shape to record from, but the aesthetic beauty of the snail and the ganglia, and the fascinating variety of depolarizing, hyperpolarizing, and bursting ganglial cells were well worth the extra effort (Fig. 5*B*). It is possible to dissect the snail buccal ganglia to the pronase stage ahead of time, if a scheduled lab time is short. Possible exercises with this preparation include depolarizing or hyperpolarizing the cells with current and then determining the amplitude and duration of action potentials. One can also examine the effects of the shape of the injected current pulse, or look for evidence of accommodation or post-inhibitory rebound.

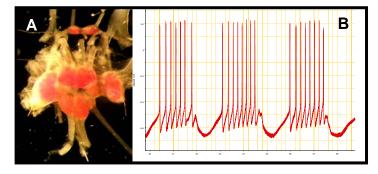


Figure 5. A. Snail buccal ganglion *B.* Spontaneous bursting patterns evident in snail neurons (images from Paul et al., 2006).

Synaptic properties and bursting neurons in snail buccal ganglia. This lab used the same preparation as above, but focused attention on the synaptic potentials that result from the connections among buccal ganglionic cells. These cells are thought to underlie feeding motor patterns in snails. The best approach to do this is to record from one of the largest cells and look for repeated patterns of post synaptic potentials (PSPs), most commonly large compound inhibitory PSPs. In some cases, it was possible to figure out which ions were responsible for the PSP by calculating the reversal potential. While participants were able to see nice varieties of PSPs, many had difficulty calculating reversal potentials. Since we did not have voltage clamps, this step required manually balancing the bridge on the amplifiers to accurately assess the membrane potential resulting from an injected current. Many of the participants were not familiar enough with the equipment to meet this challenge in the time available.

Modeling the experimental approach. In addition to all the experimental techniques, it was valuable to observe our instructors' teaching techniques. All four modeled a calm, methodological approach to the inevitable troubleshootina. This expertise probably comes from the extraordinary experience brought by the four instructors, as well as their generous personalities. Their long experience with many types of preparations and recordings - and many types of students - ensured that we were able to solve most technical problems as they arose. We all benefited from the patient way in which they helped us to think through the problem logically and systematically. In general, it was probably beneficial for our teaching skills to be on the receiving end of instruction, to be reminded of the clarity and sensitivity required for effective communication. In addition, the instructors' love of their

work and appreciation of the aesthetic beauty of the animals and recorded signals permeated every experiment. As a result, the lab atmosphere was relaxed and positive.

Network simulation, equipment, and evaluation. In our final meeting, we discussed a program called "SWIMMY," a virtual fish nervous system written in NEURON that allows students to explore central pattern generators and other neuronal activity underlying fish swimming behavior. The students can test their hypotheses about which eight of the possible 25 neurons are involved in the neural circuit driving swimming by causing or blocking action potentials (Grisham and Krasne, 2005). This program makes it possible to explore complex network concepts without electrophysiological equipment.

We also discussed equipment, sources, and some of the costs for setup of a laboratory designed to teach neurophysiology to undergraduates. Table 1 summarizes equipment and offers some suggestions for sources.

Type of Equipment	Suppliers					
Data Acquisition	ADInstruments, BioPac, Cambridge Electronic Design (National Instruments, Tucker Davis CED), iWorx Technology (TDT)					
Instrumentation	A-M Systems, Bak Electronics, Dagan, Edvotek, Gilson/Grass, labx.com, pegasusscientific.com, World Precision Instruments (WPI)					
Software	LabView (Neural Acquisition), MATLAB (Stimscope, Stimulator)					
Manipulators	Fine Science Tools, Kite, Narishige, Siskiyou Design, Stoelting					
Anti-vibration table	Kinetic Systems					
Electronic Parts	Newark, Jameco, Radio Shack					
Resistor Ladder	Edvotek					
Stereoscopes	Martin, Motic					
Puller	Sutter, Kopf, Narashige					
Wire, Glass, & Other	A-M Systems, WPI, myneurolab.com, warneronline.com, Cambridge Scientific					

Table 1. Laboratory equipment and suggested sources.

For lean budgets, some inexpensive alternatives were offered. For example, in substitution for sophisticated antivibration tables, heavy gauged steel plates can be "floated" on top of several tennis balls enclosed within a frame. Instead of buying function generators, the physics department on campus may have surplus equipment available for loan. Also, there are several websites such as www.labx.com or www.labequip.com that sell used or refurbished equipment and can facilitate equipping a lab at a modest cost.

For those who enjoy building their own equipment, there are published papers with instructions on how to build amplifiers (Land et al., 2001), a stimulus isolation unit (Land et al., 2004) and micromanipulators (Krans et al., 2006).

Crayfish can often be obtained from Atchafalaya

Biological Supply or Carolina Biological Supply. Some snails (usually *Helisoma*) can be obtained from the Connecticut Valley Biological Supply or Carolina Biological Supply.

Our last session also involved filling out evaluation forms. The tabulated results of the workshop's effectiveness and the likelihood of participants recreating the different exercises are listed in Table 2. Scores are out of 5, with 5 being high and 1 being low. Additional comments were generally very positive, with participants pleased about the instructors, the location, and the equipment. Some suggestions included having more background on theory and concepts, such as balancing bridges, adding an electric fish demonstration, and including more dissection videos.

Workshop element	Effectiveness (Mean ± S.D.)	Expected carry- over (Mean ± S.D.)
neuron models	4.14 ± 0.95	4.27±0.8
suction electrodes	4.43 ± 0.51	4.47 ± 0.64
instruments	3.23±1.09	3.36 ± 1.1
crayfish nerve 3	4.79±0.43	4.53±0.64
crayfish stretch receptor	4.71±0.47	4.47±0.64
crayfish synaptic potentials	4.14±0.86	4.20±0.77
lymnea	4.29±0.91	4.07 ± 0.96
helisoma	4.21±1.05	3.93±1.01
ADI	4.04 ± 0.5	4.19±4.04
equipment discussion	4.43±0.65	4.36±4.43

Table 2. IFEL workshop evaluation. Scores are out of a score of 5, 5 being high and 1 being low.

RESULTS

Translating IFEL experiences to home institutions – Successes, challenges, and future plans. After a fun and enriching experience at the IFEL workshop, participants hoped to put their new knowledge to good use at their home institutions. Everyone parted ways with a solid understanding of an excellent set of electrophysiology exercises and a roadmap for how to implement them.

It will take some time for most participants to follow that map to their desired ends. However, in the school term immediately following the workshop, some exercises were already successfully incorporated into courses. These included the electrical model of intracellular and extracellular recording and the demonstration of electrical recording using electric fish (Wyttenbach et al., 1999). The crayfish superficial flexor muscle prep is also being used as an experimental system for a senior research project. Some workshop participants are planning to choose a few of the cravfish and/or snail labs to incorporate into introductory neurobiology or physiology labs, while others are planning higher-level neurophysiology courses that will use most of the labs learned.

The main barrier to performing the labs for most participants is cost. Many helpful options were discussed in the workshop for those with limited budgets, and this helped participants envision possible setups that could be realized. Still, even with budget options, the array of equipment needed, particularly the micromanipulators, amplifiers, and dissecting scopes, represent a significant financial challenge to many.

Another major concern is lack of technical expertise. For those who have little electrophysiology experience, electronic equipment is largely a black box. Unfortunately, that black box has to be set up, maintained, and troubleshot, often during the lab.

A third concern involves time and energy. For many, the thought of ordering and setting up all the equipment and practicing the techniques so that they work even without the expert tutelage of our instructors and colleagues, is daunting. However, we were provided with a ready source of assistance after leaving. Post-workshop, the email list of participants and instructors has been utilized by several individuals to solicit advice regarding lab exercises, reagents, and equipment. This special collection of colleagues has proved to be a valuable resource, as described in the next section.

Birth of a new kind of learning community. Faculty Learning Communities have been created to accomplish goals such as fostering a sense of community, increasing interest in undergraduate teaching and learning, creating collaborative teaching, and nourishing the scholarship of teaching (Cox, 2004). Faculty Learning Communities have been an answer to the isolation that many faculty feel as teachers, the glaring lack of faculty development at many institutions, and the chilly atmosphere within academia.

Traditional Faculty Learning Communities are usually composed of individuals from different disciplines on the same campus at the same institution that meet fairly frequently in face-to-face interactions (Cox, 2004). In contrast, the IFEL Faculty Learning Community is comprised of individuals from different institutions that can almost never meet face-to-face. Although we come from different disciplines, we are all united under the umbrella of neuroscience. Nonetheless, it is still fair to call us a Faculty Learning Community. While at Bowdoin, some of us discovered that we teach similar courses. We exchanged opinions on textbooks and review articles, and ideas for demonstrations and laboratory exercises beyond the scope of the workshop itself. Our four day face-to-face interaction formed a professional network that remains vital and active, thanks to internet technology. This professional network maintains a flow of information and continues informal faculty development. Since our workshop last summer, this network has been used to learn methods such as lesioning hippocampi and pithing frogs, to exchange teaching resources such as virtual neural circuit programs and materials for a birdsong unit, and to offer support before upcoming reviews.

Research tends to be a collaborative enterprise, but teaching tends to be a solitary endeavor (Cox, 2004). Many junior faculty have experienced the synergy of group effort in the laboratory, but have not known its equivalent in the teaching realm. Research pursuits have strong support networks such as the Society for Neuroscience to aid

professionals in making contacts and obtaining intellectual and other resources. To strengthen and improve the endeavor, teaching also needs to build similar collaborative networks. The Faculty for Undergraduate Neuroscience can help by providing more workshops like IFEL. These workshops provide one time valuable instruction on site and then continue the faculty development by creating a network of professionals who continue to interact and support each other.



Figure 6. IFEL workshop leaders and participants congregating by Bowdoin's marine station (picture by CA Paul).

DISCUSSION

As the workshop progressed, it became apparent how much more efficient it is to learn skills at a workshop, in a group, rather than individually, at home, from a manual. Each of the participants had their own areas of expertise, so that between the instructors and our classmates, we picked up many "tricks of the trade" that would be difficult to work out on our own. Whenever there was a question, there were immediately nineteen sets of experience to draw upon to reach an answer.

At the end of our workshop, we proposed some other ideas for future workshops, such as workshops on more advanced electrophysiology, molecular neurobiology, histology, neuroimaging and fluorescence techniques, behavioral experiments, neuropharmacological techniques, computer simulation, and how to obtain funding. We IFEL veterans would love to learn more, and suspect that there are an ever-increasing number of novice neurobiology instructors and investigators who would like to join us.

In order to develop the type of faculty learning community described above, there should be a balance between time devoted to learning and practicing techniques and time for sharing teaching ideas and resources. This sharing can happen in the lab and during meals taken together, but there should be a mechanism for creating email lists and for sharing pedagogical information. Participants could be encouraged to bring electronic versions of their favorite lab exercises and class activities for possible sharing.

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