INVOLVING STUDENTS IN EXPERIMENTAL DESIGN: THREE APPROACHES

Ann P. McNeal,[1] Dee U. Silverthorn,[2] and Donald B. Stratton[3]

[1]*Natural Science, Hampshire College, Amherst, Massachusetts 01002;* [2]*Department of Zoology, University of Texas, Austin, 78712; and* [3]*Department of Biology, Drake University, Des Moines, Iowa 50311*

Many faculty want to involve students more actively in laboratories and in experimental design. However, just ''turning them loose in the lab'' is time-consuming and can be frustrating for both students and faculty. We describe three different ways of providing structures for labs that require students to design their own experiments but guide the choices. One approach emphasizes invertebrate preparations and classic techniques that students can learn fairly easily. Students must read relevant primary literature and learn each technique in one week, and then design and carry out their own experiments in the next week. Another approach provides a ''design framework'' for the experiments so that all students are using the same technique and the same statistical comparisons, whereas their experimental questions differ widely. The third approach involves assigning the questions or problems but challenging students to design good protocols to answer these questions. In each case, there is a mixture of structure and freedom that works for the level of the students, the resources available, and our particular aims.

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Many of us would like to have our students be more active in laboratories. In particular, we wish that they would understand what it means to do well-controlled experiments and to interpret data critically (1–4). However, just ''turning them loose in the lab'' is time consuming and can be very frustrating for both students and faculty. How, then, can we give students the right mixture of structure and freedom so that they take on the projects willingly and energetically, while also getting enough guidance to make the activity productive?

At a recent meeting of the APS, we three faculty from widely different institutions discussed quite distinct methods that we use to engage students in experimental design in our physiology labs. This article presents all three approaches. Dee Silverthorn teaches a separate lab course at a large public institution where students have little exposure to lab work. Labs are run by graduate students and serve 12–14 students per section. Her labs emphasize invertebrate preparations and classic techniques that students can learn fairly easily. Their challenge is to read relevant primary literature and learn each technique in one week, and then to design and carry out their own experiments in the next week. Ann McNeal teaches at a small liberal arts college where class size is small (typically 15 students). She provides a ''design framework'' for the experiments so that all students are using the same statistical comparisons, whereas their experimental questions differ widely. The lab cycle takes 3–4 weeks, with the first week providing the techniques, the second week for data-gathering, the third for statistics and data analysis, and the fourth week for presentations. Don Stratton teaches at a midsized comprehensive private university where his lecture/laboratory course in physiology (48 students in 2 lab sections of 24) is taken by undergraduate biology majors and premedical students. He wants to cover key concepts thoroughly, with a small-group format that stimulates students to design their own protocols. Therefore, he assigns the questions or problems (e.g., ''What is the effect of *X* on *Y*?''), but he challenges students to design good protocols to answer these questions. His labs are also on a two-week cycle, with the techniques introduced in a ''cookbook'' lab the first week, followed by a ''student-designed'' lab using the same techniques the second week. The vascular smooth muscle lab presented here is one of these.

STUDENT-DESIGNED QUESTIONS AND PROTOCOLS USING INVERTEBRATES

Dee Silverthorn's upper-division physiology laboratory is a two-credit substantial-writing component class. Students come from any of four large (100–200 students) lecture classes (Comparative Physiology, Vertebrate Physiology I or II, or Cell Physiology), which means they do not have common background knowledge of physiology. Most students coming into the physiology laboratory lack the background, confidence, and creativity to design well-controlled experiments without substantial instruction.

The laboratory room has six computer stations with MacLab data-acquisition systems, and there are four sections with 12–14 students each. Experienced graduate students teach two lab sections each, and they meet weekly with the course supervisor. This course is the closest that our graduate students come to teaching an independent class. They have complete autonomy in their sections within the general grading guidelines described in the syllabus. The course director monitors their teaching and grading for quality control.

The emphasis in these laboratories is on using invertebrate model systems to demonstrate basic physiological principles. A ''cookbook'' protocol is used to teach the students to measure a basic physiological parameter, such as force of muscle contraction, and then they are sent to the library to learn about the physiology and pharmacology of muscle and about the animal under study that week. Using this background, the student teams independently think of questions that they would like to try to answer, and then design and execute controlled experiments. They turn in written work weekly, and the semester ends with an oral presentation on one of their experiments.

Some parameters studied are rate and force of muscle contraction (crayfish heart, a neurogenic heart, and earthworm crop-gizzard, an innervated autorhythmic smooth muscle), electrical activity in the heart of intact animals (humans and crayfish), dye accumulation in insect Malpighian tubules, and enzyme activity (digestive tracts of various insects and crustaceans). The invertebrates used (crickets, roaches, crayfish, earthworms) are easy to obtain or raise in the laboratory and are inexpensive so that students can repeat their experiments enough times to obtain statistically significant data.

The first three weeks of the course are devoted to teaching experimental design, basic laboratory techniques such as making solutions, safety, scientific writing, simple statistics, and how to review the literature using the library and computer search engines such as Medline and Biosis. Students also learn how to use computers for data acquisition, statistical analysis, and preparing charts and graphs (Excel).

The middle 8–10 weeks of the semester are divided into 2-week blocks. In the first week of each block, students review the literature. Copies of three to four significant papers are put on reserve in the library, and the students use these as a starting point. Usually, the papers provided are from the older literature (1930–1970), and the experimental animal discussed is often different from the animal students will use in the laboratory. Generally, it is left up to the students to discover what the more recent literature has to say.

For the first week's laboratory period, students are provided with instructions for the dissection and basic methodology. They use that class as a trial session to ensure that they are comfortable with the equipment, dissection, and data collection. Between the first and second weeks, the student teams (usually 2 students per team) use the background information they have gathered to think of a question to answer and design an experiment. They know what equipment is available, and instructors try to anticipate the drugs and chemicals they are likely to request. Occasionally, the instructors will special-order a compound for someone's experiment. During this phase, students usually communicate frequently by E-mail with the instructor (graduate student) to ask questions related to their planned experiment. The final step before the second lab is to write an experimental protocol. One copy is turned in for grading, and the students use the second copy as their protocol.

In the second laboratory of each block, students execute their experiment. Most experimental manipulations test the effects of pH, temperature, pharmacological agents, or ions on the organ. The students have been making their own solutions since the beginning of the semester, and they learn to cut down on the work by making concentrated stocks and sharing solutions. To ensure that the students have created usable protocols in the early weeks, the instructors remove student lab manuals and texts from the lab room, forcing students to follow their own protocols. Each time they need to refer to an outside source to supplement their written protocol, they lose points.

The homework consists of writing up portions of a scientific paper. One semester, students were asked to write a full scientific paper each two weeks, but it was too much work for both students and instructors. Now they write selected sections up to the final experiment, where they write a full paper and prepare an oral presentation.

At the end of the semester, each team selects one of its experiments to repeat and uses the final two-week block to expand or duplicate the previous work. This allows the team to collect enough data to do a statistical analysis. Most data are analyzed using either a Student's *t*-test or paired *t*-test. In the final week of the semester, the student teams present 10-minute oral reports that they prepare using PowerPoint. All members of the team must talk. The presentations are graded both by the instructor and by peers. In addition, students must turn in individually prepared papers, written as if the research was to be submitted to a scientific journal.

Over the past 10 years the instructors have tried a variety of formats as they moved from the traditional cookbook experiments to an inquiry-based format. The only format that they would never repeat was one in which the students were introduced in detail to the anatomy and physiology of an experimental animal (the crayfish) and to a variety of experimental techniques and were then told to think of a question to answer and spend the rest of the semester designing and executing an experiment to answer that question. The students did not have enough background knowledge or understanding of research to work comfortably without more guidance. Providing more structured laboratories allows students flexibility within a limited framework and has been most successful, given the diversity of students in the class.

DESIGN FRAMEWORK AND STUDENT QUESTIONS IN AN ELECTROMYOGRAPH LAB

Ann McNeal's lab is designed to immerse students in investigation in a way that is open-ended while also being structured. First, students are introduced to the electromyograph (EMG) with a simple exercise. They also read one or more primary articles from the 1950s, when the technique was first systematized; they may also read selected papers from students of previous years. After a brainstorming session, groups of students are asked to design and carry out their own experiments. The experiments are structured as a comparison of multiple trials under two different conditions so that all students will need to use the same statistical and graphic methods of analysis—histograms and *t*-tests. These methods of data analysis are introduced during the lab. Although the experimental framework is set, the actual experimental objectives (the hypotheses to be tested) are completely open-ended, allowing full range for students' ingenuity and pursuit of questions that interest them.

The importance of the experimental structure lies in keeping the experiments finite and feasible and in ensuring that experiments generate data sets that can readily be analyzed. Too often, if students simply set off to design experiments from scratch, they may either gather too few data to be analyzed critically or design a huge experiment that does not get finished—or both!

This lab takes several weeks and involves multiple stages:

1) Introduction to principles of kinesiology (muscle use); how to operate the EMG equipment. Each group does a preliminary exercise to become familiar with recording procedures.

2) Brainstorming for ideas as a class.

3) Forming into small groups and clarifying the experimental design.

4) Running pilot experiments to see whether the design is feasible.

5) Learning computerized data acquisition.

6) Running the experiment.

7) Making tables of the raw data for each subject.

8) Learning about histograms and some simple statistics.

9) Charting histograms of the data and presenting preliminary conclusions to the class.

10) Learning more statistics—the idea behind the *t*-test for the difference between means.

11) Applying the *t*-test to the data and interpreting the outcomes.

12) Making graphs to show the summary results.

13) Presenting the entire experiment as a group oral presentation and individual written reports.

There are clearly many different ways of designing experiments like this, but to be able to analyze the data easily, the students are asked to do one specific kind of experiment:

- Compare the activity of the same muscle under two conditions,
- choose an action that is not too fast, so as not to create movement artifacts,
- measure *many* repetitions (>10) of each condition,
- alternate the two conditions (so that fatigue does not bias the results), and
- use two or more subjects, repeating the same experiment.

The purpose of using this experimental framework is so that students can see the variability in the responses within and between subjects. They learn how to analyze the data to see whether the differences in muscle activity between the two conditions are statistically significant. Although the framework is given, the actual experiment is up to the students. For example, students have chosen to examine muscle activity in

- forearm muscles during typing, supported by a wrist rest versus unsupported;
- quadriceps muscle in dancers doing a ballet movement using a specific dance teacher's image versus not using the image;
- triceps brachii muscle in push-ups with hands turned in versus facing up;
- erector spinae muscle in sitting cross legged, flat on the floor versus having the buttocks raised by a cushion; and
- activity in gastrocnemius muscle in subjects standing in high-heeled versus low-heeled shoes.

During the oral reports, all members of the group must speak. Each group must present both comparative histograms of the muscle activity under the two conditions and the statistical findings (mean, standard deviation, *t* value, and *P* value). It is in the presentation of so many visual and statistical comparisons that many students finally realize the meanings of the statistical comparisons.

This lab has two main goals—both to teach the general principles of using the EMG apparatus and to involve students in asking and answering experimental questions. To facilitate students' learning about experimental design, it is vital for the instructor to loosen up and allow that many experiments may be lacking in controls on some variables or in various other ways. If all goes well, the students themselves will realize these defects, either during the experiment or afterward, during the writing or the class's oral discussion; this is an extremely useful way for them to learn, and often a better learning experience than designing the experiment ''right'' in the first place! However, there are some designs that the instructor knows will lead to bad data. It is helpful to guide students to avoid experimental artifacts and other egregious problems.

The use of the design framework model in this and other labs has led to many very satisfying student projects. Because the students devise their own questions, the instructor does not know the answers, but this is actually an advantage. When students feel that they have creative input into the lab and feel responsible for obtaining real results (rather than results already known), they are often more attentive. On the other hand, this particular mix of freedom and structure often does not lead students to explore mechanisms and theories more deeply; Don Stratton's lab design is particularly good for achieving this aim.

ASSIGNED QUESTIONS, STUDENT DESIGN IN A VASCULAR SMOOTH MUSCLE

Don Stratton's lab is designed to give students (working in groups of 4) an opportunity to carry out experiments of their own design on tissue bath-mounted rings of rat aorta. A unique feature of this lab is that it is ''inquiry based'' and yet can be completely carried out in a single three-hour laboratory session. Each group of four students working as a team is given a single but different question to answer about a particular aspect of the physiology of vascular smooth muscle (VSM). They are given no instructions, no particular approach to take, and no set of ''step-by-step'' procedures. Their task is to think and act like scientists. They must collectively determine and carry out an appropriate strategy to answer the question. They can use whatever materials they need from the selection available at their workstation. Thus they are given the freedom and independence necessary for experimental design and yet have the assurance that they can carry the project to completion and even report on their findings to the class before the lab period ends three hours later.

Students will already have gained experience with the required preparation during the previous laboratory session. During this session, following a more traditional ''cookbook'' approach, they will have prepared and mounted in tissue baths rings cut from the rat aorta. They will also have recorded isometric contractions in response to constricting agents through a traditional strain-gauge transducer, amplifier, and recording system. Consequently, when they come to the inquiry-based lab the following week, they have had experience with preparing the tissue, know what a typical response looks like, and are ready to be challenged with something new.

The three-hour inquiry-based lab is typically timed as follows:

1) First 30 min—get the preparation up and running.

2) Next 30 min—get the question and brainstorm a solution.

3) Next 60 min—conduct the experiment.

4) Last 60 min—evaluate results, prepare overheads, and report findings.

In addition to the materials and supplies necessary for mounting and maintaining the aortic rings, students are told that they will have access to a wide variety of active agents and tissue bath bathing solutions, which will be available at their workstations. They may use any agent or combination of agents and bathing solutions in whatever order they think is necessary to successfully answer the question. The agents available to them are prepared ahead of time and are already in solution with instructions on precisely what volume to add to the tissue bath to produce a significant physiological effect. It is important to understand that the objective of this lab is not primarily to learn lab techniques but, rather, to give students adequate time to contemplate the problem, think about the ramifications of various strategies for solution, carry out the strategy of choice, and evaluate and present the results.

The agents and tissue bath bathing solutions available at each workstation are:

1) a number of agents that can produce constriction of the ring (α-adrenergic agonists, a high-molarity KCl solution);

2) a number of agents that can prevent constriction of the ring (α-adrenergic antagonists, calcium-channel blocking agents);

3) a number of agents that can produce dilation of the ring (β-adrenergic agonists, muscarinic cholinergic agonists);

4) a number of agents that can prevent dilation of the rings (β-adrenergic antagonists, muscarinic cholinergic antagonists, nitric oxide synthase inhibitors); and

5) a number of physiological saline solution (PSS) bathing solutions (regular PSS with physiological calcium levels, calcium-free PSS).

Much of the background material related to the physiology of this lab will already have been covered in the lecture portion of the course, including the mechanisms of vascular smooth muscle contraction and relaxation and the contribution of the endothelium. In addition, the lab ''handout'' includes background information on the mechanisms of action of each of the above drugs as well as the chemical makeup of the two physiological saline solutions.

Typical questions for each of the six four-member teams in a lab section might be:

Team 1: Design and carry out experiments to determine the percentage of force developed by α-adrenergic stimulation of rat aortic VSM cells that is caused by calcium influx through voltage-gated (L-type) calcium channels.

Team 2: Design and carry out experiments to determine the percentage of force developed by α-adrenergic stimulation of rat aortic VSM cells that is caused by calcium released from the sarcoplasmic reticula within the VSM cells.

Team 3: Design and carry out experiments to determine whether muscarinic cholinergic receptor and β-adrenergic receptor-induced relaxation of rat aortic VSM cells is endothelium dependent.

Team 4: Design and carry out experiments to determine whether muscarinic cholinergic receptor-mediated relaxation of rat aortic VSM cells is dependent on the synthesis of nitric oxide.

Team 5: Design and carry out experiments to determine whether β-adrenergic receptor-mediated relaxation of rat aortic VSM cells is dependent on the synthesis of nitric oxide.

Team 6: Design and carry out experiments to determine whether calcium influx through voltage-gated (L-type) calcium channels is the only route through which extracellular calcium enters rat aortic VSM cells in response to adrenergic stimulation.

Although some of these questions might seem a little sophisticated for beginning physiology students, it is surprising to find that the teams are quite adept at coming up with innovative and scientifically sound solutions. In fact, the variety of designs different teams are able to come up with for the same question is both surprising and gratifying. This latter point is particularly important, because students quickly learn that there is no single ''correct'' solution to the problem. In fact, in labs in which the same question is asked of six different teams, they learn that another team coming up with similar findings using a different approach adds corroborative evidence to their own findings. This, of course, is a common experience among research scientists.

As is the case in the EMG lab, it is wise to have teams explain their designs to the lab instructor before getting underway, to avoid significant wasted effort through a clearly faulty design.

One drawback is that there is no statistical analysis of the data collected in this lab, because *1*) the time course of vascular smooth muscle contraction and relaxation is too slow for a sufficient number of repeat runs by a single team, and *2*) because each team is running a different experiment, a ''pooled *n*'' is not possible. However, students do gain statistical experience by using computer-aided statistical analysis of collected data with many of the other lab exercises.

CONCLUSION

There are a number of features shared by all of these approaches. In all three, the basic methodology and preparation are set in advance. Students first experience the preparation through a fairly fixed or cookbook lab (although in McNeal's case, they are not issued a handout or protocol). The students' ''pump is primed'' in some way to start generating experimental ideas. Both Silverthorn and McNeal require students to read primary articles to see how other scientists have framed similar questions, whereas Stratton provides the actual question. In addition, the value of repeated experiments is stressed in all three approaches, with statistical comparisons explicitly required in two cases. In each approach, students are turned loose to brainstorm within the given structure; we do not direct them too much but are available for support. Finally, in each case we expect them to analyze and present their data like scientists, including an oral presentation that informs the whole class.

We differ in how much we direct students and how much freedom they have in framing their own questions. Stratton's lab is more directive, and he also provides more of the materials such as solutions and drugs. The payoff for him is that students provide data to reinforce key concepts. The other two labs focus more on the experimental process and the idea of making students accountable for their own experimental designs, although these designs may not be very sophisticated. Silverthorn's lab course is singular in that it is not connected to a lecture course, so she requires the students to do more library research to be sure that they are reading the background literature.

Overall, each of us has found a mixture of structure and freedom that works for the level of the students, the resources available, and our particular aims. We hope that presenting our commonalities and differences will help others in designing investigative labs that challenge students and are enjoyable for faculty.

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Address reprint requests to: A. P. McNeal, Natural Science, Hampshire College, Amherst, MA 01002.

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