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Experimental Methods in Neuroscience: An Undergraduate Neuroscience Laboratory Course for Teaching Ethical Issues, Laboratory Techniques, Experimental Design, and Analysis

Adam C. Hall[1] & Mary E. Harrington[2]
Neuroscience Program, [1]Department of Biological Sciences and [2]Department of Psychology, Smith College, Northampton, MA 01063.

We have developed and recently taught a 200 level undergraduate course entitled, 'Experimental Methods in Neuroscience'. This is a required course in an increasingly popular Neuroscience major at Smith College. Students are introduced initially to issues of animal ethics and experimentation, and are familiarized with our Animal Care Facility. Using an open field and rotarod apparatus, and the elevated plus and Barnes mazes, they conduct behavioral testing of two strains of mice, C57/BL/6J and 129S1/SvImJ, known to exhibit distinct behavioral traits. The group then employs histological techniques to prepare brain sections for observing neuroanatomical variation between strains (for example, 129S1/SvImJ mice are occasionally acallosal). In the final laboratory exercise, they assay the acetylcholinesterase activity in fore- and hindbrains from each strain.

The experiments enable the students to gain confidence in collecting data, compiling large data sets, handling spreadsheets and graphing, applying appropriate statistics, and writing accurate and concise scientific reports in journal article format. The course concludes with pairs of students conducting self-designed independent projects using the acquired behavioral, histological or neurochemical techniques.

Experimental Methods in Neuroscience is proving particularly successful as it is relatively straightforward for students to design interesting experiments, gain experience in neuroscience experimentation without excessive use of animals, gather substantial data sets, and develop skills in scientific report writing and presentation at an early stage in their neuroscience curricula. Furthermore, the course has emerged as a centralizing focus for our neuroscience program and is suitable for transfer to and adaptation by other institutions.

Key words: Experimental methods; laboratory course; behavioral neuroscience; histology; neurochemistry; independent projects; data acquisition and analysis

BACKGROUND

The neuroscience major at Smith College was created in 1997 and relied on courses that were already offered by the Psychology, Biology and Chemistry departments. After a review of the program in spring 2000, a new course was added, *Experimental Methods in Neuroscience,* which was first taught in the fall, 2001. This course was developed to address several concerns raised in our review.

One concern was that neuroscience majors did not know each other or have a sense of identity within the major. Introductory courses taken in the first year (see Table 1 outlining the major) are large lecture courses. Furthermore, the laboratory sections of Introductory Biology and Chemistry are populated by a diverse mixture of prospective science majors. We wanted to gather second-year students committing to a neuroscience major, to help them become acquainted with their cohort, and to build a sense of group membership.

We decided that a second-year laboratory focused on neuroscience techniques would encourage students in the major while they were building their basic science background through more general courses. Our goal was to offer hands-on, inquiry-based learning, and neuroscience techniques that were immediately accessible to a novice student. The course was designed so students with a curiosity for neuroscience would recognize the relevance of their chemistry and biology backgrounds. We also wanted to stress the applications of neuroscience research to important problems, so students would develop connections between research and human welfare.

Students were also unclear about how to progress from enrolling in science courses to working in a laboratory with a research mentor. Therefore, another aim was to increase the students' familiarity with the neuroscience program faculty. By direct training in introductory research skills and explicit explanation of the process by which students find a mentor to work on research projects (e.g. summer internships, special studies, and honors theses), we hoped to increase awareness of and participation in faculty-guided research activities.

Textbooks can give the false impression that all scientific territory is well explored which is particularly misleading in a field as young as neuroscience. The process of scientific inquiry and the gradual accumulation of accepted findings are often not well represented in a survey course. Thus, another goal of our new course was to demystify the process of scientific inquiry, and to help students understand how their individual research project can fit into the larger process. We wanted to emphasize reading primary journal articles, and summarizing experimental results in the format of a paper to be submitted for publication. Through these exercises, we expected to hone their reading and writing skills, and expose them to the excitement of contributing to the store of scientific knowledge.

Our present course involves several hands-on assignments. Students apply general principles from background readings and discussions about experimental design to the development of an actual experiment. The application of general principles to a specific experiment can uncover misunderstandings that would otherwise be repeated. We encourage final independent projects to be novel experiments, and for students to make a case that their research is relevant in light of previous published literature.

The course described in this article could be applied to many other settings. Using a long teaching block (three hours, twice a week), the lectures, discussions, laboratory work, data analysis, and oral presentations are intertwined. A primary characteristic is the inability to classify the course as a "lecture" or "laboratory." As it is now taught both semesters, the faculty instructor can work with small groups of students (maximum enrollment is 14) to achieve the learning objectives described below. The prerequisites for the course are Introduction to Neuroscience, General Chemistry, and one semester of Organic Chemistry (see Table 1) with the rationale that most students on a neuroscience major track will already have taken these courses by their second year.

Table 1
Required courses for the Neuroscience major at Smith College:

- General Chemistry
- Introduction to Biology
- Introduction to Neuroscience
- Physiology of Behavior
- Organic Chemistry (2 semesters)
- Cell Biology or Animal Physiology
- Experimental Methods in Neuroscience
- *Two upper level courses selected from*:
  - Molecular Neuroscience/ Neuroanatomy/ Neurophysiology
- One elective
- One seminar, special studies, or honors thesis

LEARNING OBJECTIVES

In preparation for this course, we recognized that senior Neuroscience majors were approaching upper level neuroscience classes (see Table 1) without adequate training in experimental rationale, execution, and subsequent analyses. Therefore, we identified several key learning objectives and planned a syllabus (see 'Courses' in sophia.smith.edu/~ahall) accordingly. Course assignments, detailed in the following section (Implementation and Outcomes), were designed to tackle all the following objectives:

1. Develop a critical eye for current literature, experimental design, and ethical issues in the sciences.
2. Become familiar with the proper use and handling of animals for neuroscience research.
3. Gain experience with a range of neuroscience laboratory techniques.
4. Learn tools for appropriate data acquisition and analysis.
5. Work effectively in teams.
6. Hone skills in oral presentation and writing quality scientific reports.
7. Self-design independent research projects.

IMPLEMENTATION AND OUTCOMES

Develop a critical eye for current literature, experimental design and ethical issues in the sciences.

The course begins with a general overview of the philosophy of science and of the scientific method (Derry, 1999). The class is presented with newspaper articles concerning current issues in neuroscience and attempts to discern scientific fact from pseudo-science. Students explore the distinction between experiments in a research setting *versus* a teaching laboratory, and are taught to recognize quality published articles by scrutinizing experimental design. Throughout the semester, a series of behavioral neuroscience articles are assigned and students complete critiques with questions designed to assess their understanding of the research. We discuss what constitutes an experiment (independent samples, control of other variables, randomization, and replication) with a focus on factorial design as this design is used in the final independent projects. Our exchanges culminate in a discussion of ethical issues in scientific research and with a class debate on the distinction between intuition, fraud, and deception (readings: Broad and Wade, 1994; Segerstrale, 1994).

Before conducting any experiments, the students read articles on ethical issues regarding use of animals in neuroscience research (Cohen, 1994; DeGrazia, 1994; Jackson, 1994; Singer, 1994). This gives students the opportunity to assess their position, voice their opinions, and prepare themselves for introduction to the Smith College Animal Care Facility.

Become familiar with the proper use and handling of animals for neuroscience research.

All students are required to attend a seminar on facility rules by the Director of the Smith Animal Care Facility prior to any exposure to laboratory animals. This educates the class on the required regulatory compliances, on the Smith College IACUC, on reporting concerns about the care and use of animals, and on potential risks associated with animal contact. On their first visit to the Animal Care Facility, the students are trained in proper handling of the mice and in basic facility procedures. This approach serves to allay many initial apprehensions and results in confident handling and respect for the animals during subsequent behavioral experiments (see below).

Later in the semester, through an instructional film (Hornbein, 1995), the class is introduced to the basics of aseptic technique. Readings summarize procedures for anesthesia for rodents (Davis, 2001), and a photographic guide introduces stereotaxic procedures and organization of a brain atlas (Cooley and Vanderwolf, 1978). The students later use a brain atlas to locate specific brain areas relevant to their histology and neurochemistry experiments (see below). Finally, we conduct a class discussion on seeking alternatives to use of animals in neuroscience research (see Ch. 6 in Monamy, 2000) focusing on the principle of the "3 R's": replacement, reduction, and refinement.

Gain experience with a range of neuroscience laboratory techniques.

For most students entering the major, this is their first opportunity to gain hands-on experience in a neuroscience laboratory. Exercises were selected both to serve as a sampler of neuroscience techniques (from *in vivo* to molecular) and for the relative ease in acquiring data sets for subsequent statistical analyses (see below). For all the following laboratory exercises, we work with two strains of inbred mouse, C57/BL/6J and 129S1/SvImJ (24 of each, divided equally between male and female). We chose these strains since previous studies have described their distinct behavior (Crawley, 2000) and determined that they have different neuroanatomical features (*e.g.* 129S1/SvImJ are occasionally acallosal; Lipp and Wahlsten, 1992). For instance, from previous reports (Contet et al., 2001; Montkowski et al., 1997), it was expected that the C57/BL/6J mice would demonstrate higher levels of locomotion in the open field as they are typically less anxious compared to the 129S1/SvImJ strain. Furthermore, these mice are readily available (Jackson Laboratories, Bar Harbor, ME), easily identified, and their maintenance is straightforward (housed in pairs by gender on a 12:12 L:D cycle and fed *ad libitum*). All the procedures in this course were approved by the Smith College IACUC.

Behavioral Neuroscience Laboratories: The students are assigned background readings on behavioral neuroscience experiments (Crawley, 2000; Brown et al., 2000) with an emphasis on characterizing mouse behavior for studying mutant (e.g. transgenic, knock-in/-out) animals. As an introduction to quantifying mouse behavior, the class scores behaviors (e.g. climbing and rearing, urination and defecation, time in selected quadrants) of a mouse in an open field test from a pre-recorded video. We use these initial measurements and readings from an accompanying text (Martin and Bateson, 1993) to introduce the concept of inter-rater reliability in data collection, and discuss the importance of using strict criteria when scoring behavior. Pairs of students then measure the locomotor activity for an equivalent number of male and female mice of each strain. The experimenters adhere to a strict protocol, cleaning the open field between trials, placing the mouse in the center of the field (a standard animal housing cage, 40 x 40 cm) and monitoring the activity (HVS Image Video Tracking System, HVS Image, Buckingham, UK) over two min. The HVS Image tracking system reports a number of variables (e.g. total path length, time in center *vs* periphery of field) for subsequent analyses. The resulting data sets are used in a preliminary laboratory report (only Methods, Results with figures) to compare the activity between strains/sex using boxplots, histograms to examine distributions and calculations of statistical significance using Student's t-test (Figure 1).

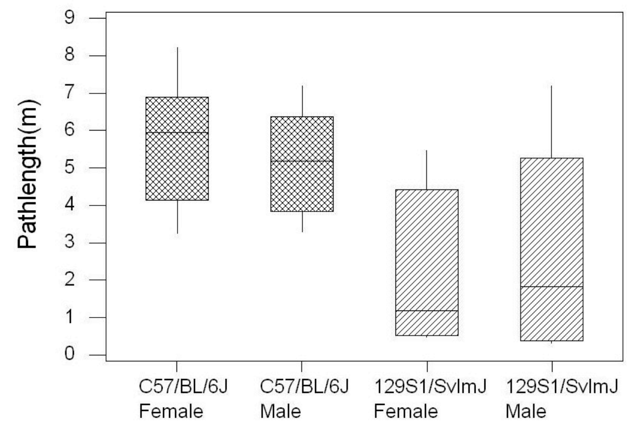

Figure 1. Open field activity for two mouse strains. Locomotor activity is monitored for 2 min and pathlength (m) measured for mice of different strains/sex (n=6 mice in each of the four groups). C57/BL/6J mice demonstrate greater levels of activity although there is no difference between males and females within strains (p>0.05).

Neuromuscular coordination of the mice is measured using a rotarod test (San Diego Instruments, San Diego, CA). Pairs of students test groups of mice for latency to fall from the apparatus after the rotarod is accelerated in the first minute from 0-18 RPM and then maintained at a constant speed. The latency data are compared with the results from the open field locomotor activity trials, and correlation analyses performed. Methods and Results sections (including figures and legends) from this comparison comprise their first assessed report. Typically there are no differences between the strains in their performance on the rotarod as described previously (Contet et al., 2001).

As a measure of the relative anxiety levels of the different strains/genders, students conduct elevated plus maze testing (Figure 2). These experiments involve placing mice in the center of a raised platform in the shape of a cross with two 'closed arms' and two 'open arms' (Columbus Instruments, Columbus, Ohio). Mice are allowed to navigate the apparatus for 5 min and their movements are tracked using the HVS Image tracking system.

In the elevated plus maze a high number of entries to the open arms implies a greater level of exploration and minimal anxiety. Conversely, a reluctance to leave the closed arms is suggestive of heightened fear and emotionality. Students research previous literature to design their hypotheses, conduct elevated plus maze trials for all mice, and pool the class results to generate data sets of 'time spent in open *vs* closed arms.' From observations of total locomotor activity (Figure 3) their studies typically confirm heightened anxiety levels for this particular 129 substrain as previously reported for 129 mice (Contet et al., 2001) (although number of entries to the open arms typically does not differ between strains).

Thus, students become aware of the difficulty of separating measures of anxiety from measures of locomotor activity.

Figure 2. *S*tudents observing behavior of a C57/BL/6J mouse in the elevated plus maze. Video-tracking system is positioned above the apparatus to monitor the animal's exploration of the maze.

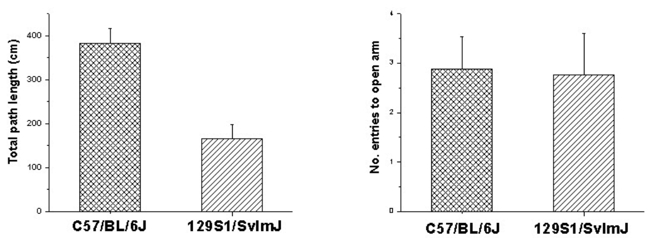

Figure 3. *S*train differences in behavior on the elevated plus maze. C57/BL/6J mice exhibited significantly ($p<0.05$) more locomotor activity but a similar tendency to venture into the open arm on an elevated plus maze over a 5 min trial. Histograms represent means and standard errors of the mean for n=12 mice for each strain.

For a final behavioral testing apparatus, we give a demonstration of a Barnes maze (built in-house) consisting of a circular board (122 cm diameter) with 40 holes on the periphery, one of which leads to a plastic escape box. The Barnes maze (Barnes, 1979) is a learning task, and we use the spatial version with visual cues on a wall round the circumference of the maze to assess the relative learning ability of the two strains. For a habituation period the mice are placed in the escape box for 30 s. During the testing period, they navigate the maze and the time to find the escape box is recorded. An advantage of this maze is that it provides a measure of spatial memory without the need for food deprivation or forced swimming. This apparatus often proves to be a popular option in the design of final independent projects (see below), although, again, there are no obvious differences in the performances of the two strains on this apparatus (see Contet et al., 2001).

Histology: Mouse brains from C57/BL/6J and 129S1/SvImJ strains are prepared in advance by the instructors by CO_2 asphyxiation, decapitation, and removal of the brain into a 10% formalin solution. A day before the laboratory, brains are transferred to a 30% sucrose solution. Students are trained to block the brains and position the tissue on a standard microtome for cutting. Tracking the location using a mouse brain atlas, they proceed to cut 40 µm coronal or sagittal sections, and mount 5-6 sections per glass microscope slide. Mounted sections are stained using cresyl violet, and coverslipped with Permount for viewing with a light microscope (background provided by readings from Ch. 14 in Barker, 1998). The class is taught to recognize landmarks (e.g. the habenular nucleus in sagittal sections) for verifying the location of their sections, and thereby to reconstruct a 3-D image of the brain and to distinguish neuroanatomical differences between the two mouse strains. As noted above, 129S1/SvImJ mice can be acallosal (Lipp and Wahlsten, 1992), which is most easily observable in sagittal sections (Figure 4).

Students generate data sets for the two strains comparing length and area of the corpus callosum at equivalent positions in the brain. Severe agenesis is observed in a proportion (*ca.* 30%) of 129S1/SvImJ mice. Of interest is that a recent paper indicates the BTBR T/+ tf/tf strain may show callosal agenesis with greater reliability than the 129S1/SvImJ substrain used in our laboratory (Wahlsten et al., 2003).

Neurochemistry: For this laboratory we use an adaptation of the acetylcholinesterase enzyme assay described in "Laboratory 2, An introduction to neurochemistry" from *Discovering neurons: the experimental basis of neuroscience* (Paul, 1997). Brains are removed, dissected, homogenized and placed on ice by the instructors immediately before the exercise. Rather than compare enzyme activity in a variety of brain regions as described in Paul (1997), we adjust the protocol working with four samples: C57/BL/6J and 129S1/SvImJ forebrain and hindbrain, and collate data from the whole class. Although enzyme activities between strains are equivalent, as expected there are substantial differences in acetylcholinesterase activity in forebrain *vs.* hindbrain.

Learn tools for appropriate data acquisition and analysis

The course is designed to teach accurate data collection and use of suitable statistical tools to analyze experiments. Collecting data sets from the two mouse strains from the behavioral, histological, and neurochemical laboratories provides a unique opportunity for the class to test hypotheses and to postulate links between anatomy and behavior. We stress that it was essential to keep clear and thorough laboratory notebooks (described in Ch. 5 of Barker, 1998) throughout the course. Students familiarize themselves with programs for acquisition and data processing, particularly HVS Image software, NIH Image (http://www.scioncorp.com/), Microsoft Excel, and Minitab. We find it most effective to teach the statistical component in a computer classroom where Excel spreadsheets of pooled results can be projected and scrutinized by the whole class. This component includes (1) examining distributions with histograms, with measurements of average (mean, median) and spread (quartiles, standard deviation), and using boxplots (2) correlation and regression analyses for interpreting scatterplots (3) inferential statistics for hypothesis testing of statistical significance (parametric *vs* non-parametric, t-tests, and ANOVAs). While this encompasses a wide range of statistical techniques, this component is taught from sections of a helpful textbook (Moore and McCabe, 1999) without attempting to include detailed probability theory. In this way, students acquire the tools to perform basic statistical analyses and are advised to take further statistics courses. The advantage of developing neuroscience laboratories that generate substantial data sets with relative ease is most apparent from the enthusiasm the class expresses in applying these statistical tools to test their hypotheses.

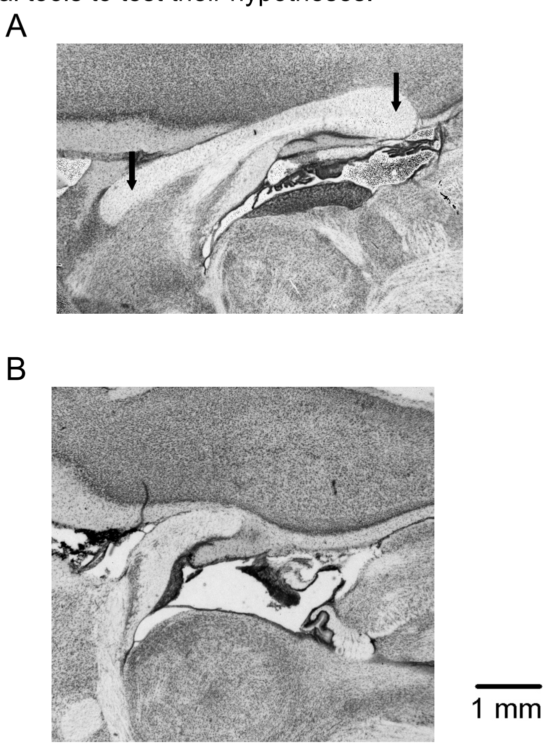

Figure 4. Agenesis of the corpus callosum in 129S1/SvImJ mice. Cresyl-violet stained sagittal sections of brain from (A) C57/BL/6J and (B) 129S1/SvImJ mice. Arrows indicate location of corpus callosum. Severe agenesis is observed in a proportion (*ca.* 30%) of 129S1/SvImJ mice.

Work effectively in teams

One of the goals of this course is to develop camaraderie amongst the incoming majors and thus, whenever possible, we attempt to combine their class efforts. All the laboratory exercises including the final independent projects (see below) are conducted in pairs. Data sets are typically generated by pooling results that has the added effect of raising awareness for accurate data collection. Classroom work is typically conducted through open discussion, group debate or presentations that serve to encourage a collective identity. Furthermore, the course is supported by two undergraduate teaching assistants, both Neuroscience majors with previous experience of the experimental protocols.

Hone skills in oral presentation and writing quality scientific reports

Students are assigned two opportunities to give oral presentations to the class. In the first assignment, pairs design and deliver PowerPoint (Microsoft) presentations based on a summary of a peer-reviewed article on behavioral neuroscience. After feedback from the instructor and the class, this exercise is considered preparation for the presentation of their final research projects (see below).

Throughout the course, we place considerable emphasis on learning to write a quality scientific report. For each laboratory exercise, we require and correct an initial draft, and then assess their revised manuscripts. In their first reports, students focus on writing Methods and Results (with figure legends) sections only. As they gain experience, later reports incorporate Introduction and Discussion sections. For their final project they write a full report adhering to *Journal of Neuroscience* guidelines. Again, the use of a fully equipped computer classroom is invaluable for in-class writing assignments when revising student drafts and demonstrating the appropriate format and style of each section.

Self-design independent research projects.

In the last three weeks of the semester, students research and select independent projects based on any of the techniques (behavioral, histological, or neurochemical) that they have learned during the course. Projects involve presenting a hypothesis, designing and conducting suitable experiments to test this hypothesis, data analysis, writing a full report and presenting their findings to the class. The students have carefully researched the background literature and thus design projects that are not replicative (unless justified). The majority chose to work in pairs but occasionally there are teams of up to four. There has been a wide range of final projects; for example, one group observed the impact of introducing novel objects on behavior of mice on the elevated plus maze, another assessed the effects of different visual cues on spatial learning in the Barnes maze, and another studied relative areas of corpus callosum in sagittal brain sections in the two strains. The final project draws on all aspects of the semester's teaching and has been assessed by the students to be a rewarding culmination to the course.

EVALUATION

We have now offered this course three times, so it is still premature for a full assessment of its impact. However, outcomes have been consistently positive, as assessed by the instructors as well as the students. Students developed a critical eye for quality scientific pursuit which was best demonstrated by their ability to detect flaws in published papers. Scientific inquiry involves participation within a community of peers who share standard for types of evidence admitted to a debate. Our course was able to build such a community and to encourage informed debate.

Students were able to master the techniques presented reasonably quickly, as demonstrated by their ability to design independent projects. The behavioral tests were probably the most effective in this regard, as determined by the number of students applying these techniques in their final studies. We recognized that we could not introduce students to the entire range of techniques encountered within the field of neuroscience. Our students will take advanced laboratory courses as well (see Table 1) in which they will gain exposure to a wider range of techniques.

All students completed the required training program for the use of laboratory animals at Smith College, and had direct experience working with mice in the laboratory. They ended the course with a strong background in ethical considerations for animal experimentation and in proper use and handling of animals in neuroscience research. Many preconceptions had been challenged by classroom discussions and experiences.

We judged that we succeeded in building a sense of camaraderie among the neuroscience majors. Students worked effectively in teams to accomplish laboratory exercises and final projects. End-of-semester social events and field trips also helped to build group cohesion. An unexpected benefit was that this course began to serve as a centralizing focus for the neuroscience program. Upon completion students were adept at organizing data into a database, graphing results, and performing basic statistical analysis. The use of these two strains of mice was helpful, in that behavioral differences between the strains were robust, although results showed some variability. This helped students grasp the need for inferential statistical tests, while statistically significant differences in the behavioral, neurochemical, and histological measures gave a sense of reward after completion of the data analysis.

The contrast between the initial attempts at writing reports in journal article format to their final papers gave a clear indication that we were extremely successful in developing better scientific writing skills. Grades on written journal article critiques showed steady improvement during the semester, demonstrating an enhanced ability to comprehend journal articles, and to extract the key information. Most students were already exposed to oral presentation software, but definitely improved their public speaking skills and ability to relay scientific material.

CONCLUSION

Our new course, *Experimental Methods in Neuroscience,* addressed the needs of our Neuroscience program and would probably be suitable for adaptation at many other institutions. The purpose of this course was to bring together sophomore neuroscience majors and to establish basic research skills. While a full assessment of the impact of this new course is not yet possible, we are confident that it has dramatically improved scientific writing skills in these students. A sophomore recently commented that she now realizes the importance of reading primary journal articles, and wishes she had done more of such reading sooner in her undergraduate career.

Since its introduction in 1997 the number of students majoring in neuroscience at Smith College has risen from four to over 50 (juniors and seniors). ‘Experimental Methods’ has provided an avenue from the Introductory courses into the major and acquainted incoming students in the necessary research techniques to embark upon the upper level courses. Of the students who have taken this course, 67% have elected to work in research laboratories either at Smith College (special studies and Honors) or in summer internships elsewhere. Several students attended a regional meeting to present their final research projects from the course, and 14% have published work from subsequent faculty-guided research projects.

The course could be improved by the inclusion of more molecular techniques in laboratory exercises. Indeed, while maintaining the aims of this course, the laboratory components could always be modified substantially to accommodate approaches better suited for existing facilities or curricula of individual institutions. The two three-hour afternoon time blocks have proved appropriate for teaching both laboratory and lecture components of the course. However, we have encountered a problem in that mornings are unavailable due to commitment to lecture courses and occasionally there are scheduling conflicts with afternoon labs. For most cases, this conflict is avoided by offering the course both semesters. Finally, we would benefit from access to instructional videos covering techniques such as construction of transgenic mice. We will continue to adapt *Experimental Methods* to provide a useful grounding in research techniques for our incoming majors, and welcome any suggestions from readers to develop the course further.

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Address correspondence to: Dr. Adam Hall, Biology Department, Smith College, Northampton, MA 01063 Email: ahall@smith.edu