ARTICLE

Developing a Project-Oriented Introduction to Neuroscience Lab at Hope College

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The *Introduction to Neuroscience* course at Hope College includes a three-hour laboratory period each week. Seven of the fifteen weeks of the lab are used for a lab project that is focused on understanding the effects of gonadal hormones on brain and behavior. Students perform ovariectomies and implant sham, estradiol, or testosterone capsules in rats and then carry out five experiments: 1) Sexual Behavior, 2) Spatial Learning using the Morris Water Maze, 3) The Size of the Sexually Dimorphic Nucleus, 4) Phosphorylation of NMDA Receptors, and 5) Long Term Potentiation in Hippocampal Slices. The experiments are designed to provide the students with experiences at different levels of neuroscience, while improving their skills in statistics, using the primary literature, and scientific writing. The students generate interesting and statistically significant data which they summarize in a journal style lab reports. Using a Self Assessment of Learning Gains tool, we learned that students perceive the lab project improves their ability to A) pose questions from more than one disciplinary perspective that can be addressed by collecting and evaluating scientific evidence, B) learn about complex science problems that require insight from more than one discipline, C) extract main points from a scientific article and develop a coherent summary, and D) write reports using scientific data as evidence. Based on our results, we believe an extended lab project in an introductory neuroscience course can be used to engage students in neuroscience topics and help them develop the skills and habits of neuroscientists.

Key words: introductory neuroscience course, lab project, liberal arts college, ovariectomy, sex steroids, spatial learning, long-term potentiation, sexually dimorphic nucleus, hippocampal slice, self assessment of learning gains, NMDA receptor phosphorylation, sex behavior

THE COURSE

Students interested in neuroscience at Hope College are encouraged to enroll in *Introduction to Neuroscience*, a four-credit sophomore level course with no prerequisites, which is the first course in the Neuroscience Minor program at Hope. The development of this program was described in detail in a prior paper published in JUNE (Chase et al., 2006). In this paper, we focus on a semester long lab project in the introductory course, which is designed to help fulfill the objectives of that course:

1. To learn the fundamental principles of neuroscience (from the molecular level to the systems/cognitive level).
2. To learn multiple experimental techniques used by neuroscientists.
3. To learn how to develop scientific hypotheses and how to design experiments to test those hypotheses.
4. To learn how to critically evaluate scientific data.
5. To gain an appreciation for the interdisciplinary nature of neuroscience.

In considering how the lab would contribute to meeting the course objectives, we considered several approaches for the lab such as inquiry-based student projects (Woodhull-McNeal, 1992; Sundberg and Moncada, 1994; McLean, 1999), having a single project for the entire semester (Goyette and DeLuca, 2007) and using computer simulations, as has recently been reviewed by Bish and Schleidt (2008). We decided, however, to use a combined self-contained labs and project lab approach, in which students would work in teams to carry out a long-term project that would introduce the students to many aspects of neuroscience research (Leonard, 2000; Switzer and Shriner, 2000) similar to the approach used by Hall and Harrington (2003) in their neuroscience laboratory course at Smith College.

THE LAB PROJECT

The laboratory for the *Introduction to Neuroscience* course meets for 15 weeks. The students are divided into six groups with three or four students in each group. Using the class list, the instructors set up the groups so that, as far as possible, each group will consist of students majoring in different disciplines at Hope. The laboratory consists of the project and eight self-contained labs. The self contained labs are designed to expose the students to experimental topics and techniques that illustrate the different levels of analysis with which neuroscience is concerned. The first week is primarily concerned with statistics using student reaction times as data. Three labs utilize Crawdad (Wyttenbach et al., 1999) to investigate resting membrane potentials, action potentials and stretch receptor responses. In the remaining four labs, students perform a neurological exam, complete a sheep brain dissection, examine aspects of smell and taste, and are introduced to categorical perception of language (developed by Dr. Sonja Trent-Brown of the Hope College Psychology Department).

The remaining seven weeks of lab are used for the semester long project. We developed five specific objectives for the project:

1. To develop the skills and habits of a research neuroscientist.
2. To gain insight into the value of doing multiple levels of neuroscience research (molecular to behavioral) in order to address a single scientific question.

3. To gain experience with experimental techniques commonly used by neuroscientists.
4. To gain experience working collaboratively with other researchers with different disciplinary strengths.
5. To gain experience with all aspects of scientific communication including reading the scientific literature, data analysis and interpretation and writing of research results in a formal way.

This project was conceived when the core members of the neuroscience program met and discussed ways to incorporate their research strengths into the lab curriculum. Specifically, they focused on the experimental techniques in which they had expertise. They then discussed various projects that encompassed many of those techniques and would be interesting to students while considering the equipment available at Hope. With this in mind, a project entitled *The Effects of Gonadal Steroids on the Structure and Function of the Rat Brain* was developed. Students work in multi-disciplinary teams to carry out a multi-week research project with rats aimed at demonstrating the effects of gonadal steroids on brain structure, physiology, and behavior. In addition to specific experimental techniques, students gain experience in working with living experimental animals, keeping a lab notebook, statistical analysis of data, reviewing the primary literature, data interpretation, and scientific writing. At the end of the semester, each team submits a final journal style scientific manuscript reporting on the results and their significance.

The project begins in the fifth week of the semester when students ovarectomize and implant hormone capsules into female Long Evans rats. The rats are divided into three groups of eight rats each: 1) Ovariectomized and implanted with an empty Silastic™ capsule (OVEX), 2) Ovariectomized and implanted with a Silastic™ capsule containing crystalline estradiol benzoate (OVEX-E), and 3) Ovariectomized and implanted with a Silastic™ capsule containing crystalline testosterone propionate (OVEX-T). The schedule for the rest of the project is:

Week 10 - Rat Sex Behavior
Weeks 12 and 13 - Spatial Learning
Week 14 - Volume of the Sexually Dimorphic Nucleus
Weeks 14 and 15 - Phosphorylation of NMDA Receptors
Week 15 - Long Term Potentiation (LTP)
Week 16 - Final Data Analysis/Writing

This project has evolved over the last three years with refinements being made in the experiments each year. In 2008 we added the LTP component and increased our emphasis on statistics. For 2010, we will add an analysis of rat body weight and rat hair growth, and expand the role of the students in the last three experiments while further working out some of the “kinks” in those labs.

EXPERIMENTAL METHODS AND RESULTS

Ovariectomy: As the ovariectomy and Silastic™ capsule implantation lab is the first experience that many of the students have using live experimental animals, we spend part of the prior week’s lab discussing vertebrate animal use, including ethical considerations and animal care and use regulations. We inform the students about the process by which the project was approved by the Hope College Animal Care and Use Committee and explain how any concerns about animal care and use the students may have can be brought to the attention of the Committee. We also show them a 45-minute video that demonstrates the entire surgery, including the use of anesthesia, analgesia, aseptic techniques, isolation and removal of the ovaries, implantation of the capsules (sc. at the back of the neck), using sutures and wound clips to close incisions and post-surgical monitoring. These procedures are also explained in detail in the lab handout and are summarized in a pre-lab discussion immediately prior to surgery. During the lab, the instructor is assisted by two other faculty members with experience in the procedure so that each student lab group has ready access to faculty assistance if needed.

Rat Sex Behavior: After allowing five weeks for the effects of ovariectomy and steroid treatment to be manifested, students observe receptive sex behaviors in the rats (Kuehn and Beach, 1963). Each experimental rat is placed in a testing arena with a sexually experienced male rat for 20 minutes and the students score attempted mounts by the stud male, and the students record the lordosis number and the quality of the lordosis based on the degree of back arching (range 1-4). The mounts data are analyzed by One Way Analysis of Variance (ANOVA) followed by Tukey tests for comparisons of means using SYSTAT 11 (for which the students are provided a detailed handout). The data for this experiment from 2008 are given in Table 1, which demonstrates that hormone treatment has a significant effect on mounts. Lordosis number and quality are also affected by hormone treatment.

Group	Mounts	Lordosis Number	Lordosis Quality
OVEX	2.1±1.1[a,b]	0±0	0±0
OVEX-E	6.9± 2.3[a]	6.0±2.1	2.4±0.5
OVEX-T	1.2±0.4[b]	0±0	0±0

Table 1. Results of the Reproductive Behavior Observations. Ovariectomy and steroid treatment had significant effects on the number of mounts, the number of lordosis events and the lordosis quality. Shown are the means±SEM. Values within a column not sharing a superscript letter are significantly different at $p<0.05$.

Spatial Learning: For the determination of spatial learning, students are introduced to the Morris Water Maze (Mulder and Pritchett, 2003) and instructed to determine the time to the raised platform and to trace the route followed by the rat on a scale drawing, doing this twice for each rat. The distance on the drawing is then measured but not scaled up. Each lab group is then assigned one day in the following week to train the rats in the maze and record time and distance traveled. In the end, the rats are trained each day of the week, with the exception of the weekend. During the next lab period, the final measurements with a hidden platform are made and the rats are then tested twice with a visible platform in a new location to determine if factors such as motor performance might be affecting the data. The students record the data on an Excel file on a computer in the lab and the data are analyzed by Two Way ANOVA with repeated measures (non-visible platform) or One Way ANOVA (visible platform). As shown in Fig. 1, all three groups of rats exhibit spatial learning, as indicated by improvements in the time to reach a hidden platform. There is no effect of treatment on this variable. Similar results are seen with the distance to hidden platform data. In addition, there is no effect of treatment on time (Fig. 1 inset) or distance to the visible platform, which indicates no differences in motor performance between treatments.

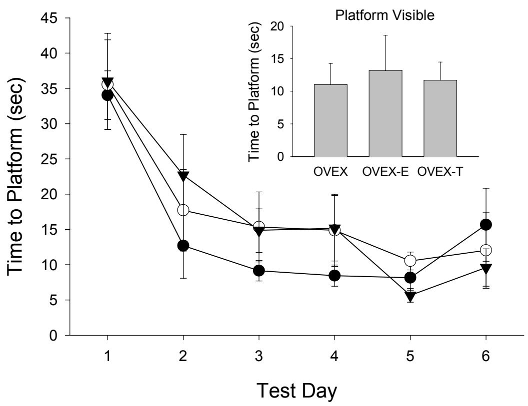

Figure 1. The effects of ovariectomy and steroid treatment on spatial learning as tested by the time to the hidden platform in the Morris Water Maze test and on motor performance in the time to the visible platform (inset). Data shown are means±SEM. ● = OVEX, ○ = OVEX-E, ▼ = OVEX-T. ANOVA of the time to hidden platform indicated a significant ($p<0.001$) effect of test day but no significant effect of treatment or interaction between test day and treatment on time to platform. ANOVA of the time to visible platform data indicated no significant effect of treatment.

Size of the Sexually Dimorphic Nucleus of the Preoptic Area (SDN-POA): To determine the volume of the sexually dimorphic nucleus (Gorski et al., 1980), the faculty sectioned and Nissl-stained brains of a subset of the rats. Students measure the diameter of the SDN-POA in serial 40 μm sections for each rat using an inverted, projection microscope. The students estimate the volume of the sexually dimorphic nucleus for each rat from these data. The data are analyzed with One Way ANOVA. Although there are interesting trends in the data (Fig. 2), there is no significant effect, perhaps due to the low number of replicates or the age of the rats at the time of ovariectomy.

Phosphorylation of NMDA Receptors: Rosenblum and colleagues (1997) have demonstrated that phosphorylation of the NR2B receptor subunit of the NMDA receptor occurs as a result of the induction of LTP. Therefore, as an indirect measure of LTP in different brain regions, students are asked to use western blot analysis to determine the extent of phosphorlylation of the NR2B subunit. The students are provided with homogenates of the hippocampus, cortex and cerebellum from three rats from each group for this study. The students determine the protein concentration of each sample and then use those values to load an equal amount of protein into each lane of a SDS polyacrylamide gel. Following electrophoresis, the proteins are transferred to a nitrocellulose membrane for chemiluminescent detection and the Scion Image program is used to quantify the amount of phosphorylated and non-phosphorylated NR2B subunit of the NMDA receptors.

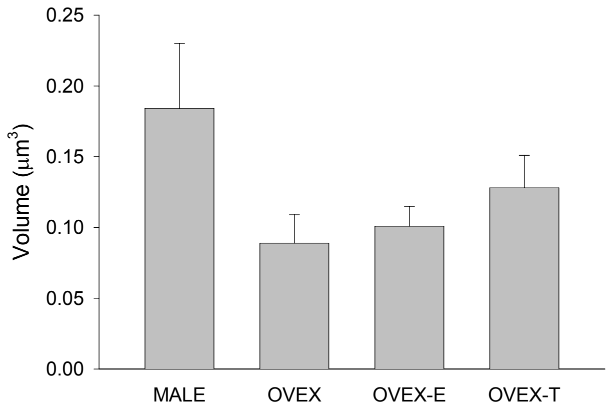

Figure 2. The effects of ovariectomy and steroid treatment on the volume of the sexually dimorphic nucleus as compared to untreated male rats. Data shown are means+SEM. ANOVA indicated no significant effect of group.

The students then determine the ratio of phosphorylated to non-phosphorylated receptors and analyze the data with One Way ANOVA and Tukey tests. As shown in Fig. 3, estradiol and testosterone significantly reduce the ratio in the cortex and hippocampus. Testosterone, but not estradiol, significantly increases the ratio in the cerebellum.

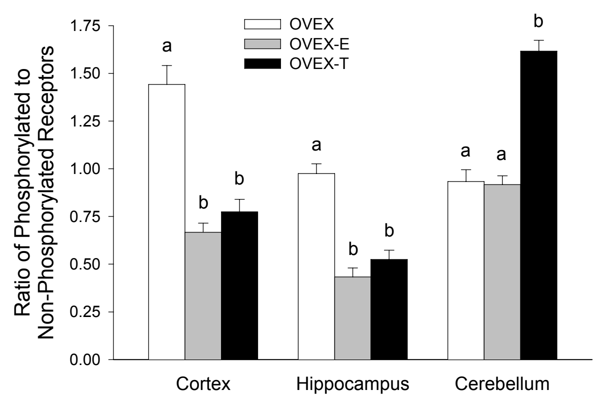

Figure 3. Effects of ovariectomy and steroid treatment on the ratio of -phosphorylated to non-phosphorylated NMDA receptors in the cortex, hippocampus and cerebellum. Data shown are means+SEM. For each brain region, data not sharing the same letter are significantly ($p<0.001$) different.

Long Term Potentiation (LTP): In the final experiment, the students investigate the effects of ovariectomy and steroid treatment on LTP expression in hippocampal slices. The faculty set up the slice chambers with the hippocampal slices and the students measure evoked EPSPs (0.1 Hz) before and after a tetanic stimulation (100 Hz for 1 sec). The students determine the slope of the EPSPs and then analyze the data with Two Way ANOVA with repeated measures. Tetanic stimulation led to an increase in EPSP slope in hippocampal slices from all three experimental groups but there was no significant effect of treatment on LTP (Fig. 4). However, students only were able to make measurements from three slices from each treatment group. We hope to gain more statistical power in the future by increasing the number of slices analyzed.

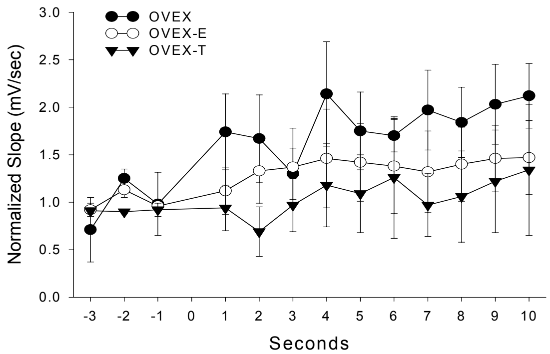

Figure 4. Effects of ovariectomy and steroid treatment on the LTP in hippocampal slices. Data shown are means±SEM. ANOVA indicated a significant ($p<0.005$) effect of time but no significant effect of treatment or interaction between time and treatment.

HIGHLIGHTS AND CONSIDERATIONS

For each component of the project we recorded what were some highlights or success and some issues that need to be considered or problems that occurred. In general, the students were enthusiastic about the project with periods of frustration when procedures took longer than expected or were problematic. Not surprisingly, the students loved the opportunity to be surgeons. In part because of the use of the video, the surgical procedures have been very successful even though for most students it is a new experience. The surgery provides experience in surgical skills and animal care. The students are fascinated to learn that they have the ability to carry out a surgical procedure on a living animal. To see that animal behaving normally the next day builds confidence. Ethical issues related to the use of animals in research are made real. We have found that most students become excited and enthused about the lab project after completing the ovariectomy lab, leading to significant student engagement with the project (see student comments in the Assessment section of this paper).

The success of the ovariectomy lab depended on having faculty who were experienced in animal surgical procedures, careful training of the students (through the use of the video) and an emphasis on animal welfare concerns/ethical issues. Our institutional policy allows students who have ethical concerns to opt out of labs in which animals are used, and in 2008 one student opted out of doing the surgery (but attended lab) because of those concerns. Most students had not previously handled rats, so time was needed to allow them to gain experience and confidence in doing so. Most students were surprised at the thickness of rat skin and the force needed to insert needles for injections of anesthetics and the analgesic. Students were overly cautious in making incisions and manipulating tissues and in some cases needed to be shown that one could cut through the skin and separate muscle layers quickly. Students also needed to be reminded repeatedly about the importance of using aseptic techniques. We observed that the groups in which the students had clearly delineated for themselves (as outlined in the lab handout) specific jobs and worked as a team finished the procedures more quickly and with less apparent stress than did other groups. In 2008, a faculty member needed to reapply wound clips to one rat, but no other procedural complications occurred.

The experiments on reproductive behavior provided the students with a good introduction to the analysis of animal behavior and related directly to lectures on the organizational and activational effects of gonadal steroids on the brain. The results demonstrated very clear effects of gonadal steroids on behavior (Table 1) and the behavior itself is of interest to students. In addition, the lab is very inexpensive. Although this experiment was simple for the students, we had to twice hormonally prime the female rats prior to being ovariectomized and allow each rat access to a male rat for 20 minutes in order to give both the male and female rats practice with the desired behaviors. The OVEX-T rats exhibited higher levels of aggression than the other female rats and occasionally had to be removed from the observation cage because of fighting with the male rats. When placed with steroid-primed female rats (OVEX-E) the OVEX-T rats tended to show mounting behaviors rather than typical female receptive behaviors (lordosis). The students had to occasionally be asked to refrain from making non-scientific comments about the behavior they were observing. Finally, as very little receptive sexual behaviors were exhibited by the OVEX or OVEX-T rats during the 20 minutes of observation, students learned that behavioral observation research is not always exciting.

The experiment with the Morris Water Maze provided another opportunity for behavioral observations using a test about which the students had often previously read. In addition, students found this experiment on spatial learning inherently interesting given reports in the mainstream media on the subject of spatial memory differences between women and men. Students have a more concrete understanding of spatial leaning after analyzing the data and noting the marked improvement in performance over time (Fig. 1). A variety of computerized imaging systems are available for use with the Morris Maze, but the experiment can be performed quite well with just a stopwatch, paper, and pencil, although the distance measurements are not as accurate. The experiment does require a significant amount of dedicated lab space for one week, which may cause concern if the space is shared with other courses. In addition, it was difficult for some students to schedule a two-hour period outside of lab time for the measurements and it was not possible for these students to work in their lab group on the measurements. Finally, we found that requiring students to enter their data into an Excel spreadsheet at the conclusion of each, training run (time and distance) greatly simplified the final data analysis process.

The sexually dimorphic nucleus experiment was designed to give students experience looking at brain sections and experience in finding and measuring a particular nucleus. Time, sample and equipment limitations led to only a subset of the students doing these measurements, which they then shared with the rest of the class. Furthermore, the data exhibited trends suggesting a steroid effect, but no statistically significant differences (Fig. 2); although, these trends did allow the students to consider how brain anatomy may be under gonadal steroid control. We plan to improve this experiment in the future by increasing the number of samples and having every student analyze the SDN-POA. We would also like to have the students section and stain the brains, but we need to determine how to fit that activity into the semester schedule.

During the NMDA receptor phosphorylation experiment, the students are introduced to western blots, a modern biochemical method used in neuroscience, and a level of analysis not used at any other point in the lab. The effects of the steroid treatment and the different response of different brain areas to steroid treatment are readily apparent (Fig. 3) as are differences in the expression of NMDA receptors in different parts of the brain (data not shown). The lack of prior experience with biochemical techniques by many of the students led to variability in the time needed for the protein determinations from year to year and to inconsistent performance in loading of the gels. In the future, we plan to determine the protein concentrations for the students in advance of the lab so that they can focus on loading and running their gels and transferring their samples to membranes. In addition, we will be obtaining a gel documentation system so that each group of students will be able to quantify their own blots.

Dr. Chase's prior experience with LTP measurements in hippocampal slices led us to add this experiment in 2008. As we were discussing LTP in lecture during the same week, the experiment allowed the students to better connect lab and lecture material while giving the students experience with another electrophysiological recording paradigm and a widely used preparation, the hippocampal slice. The students were able to observe LTP in the slices (Fig. 4) although no steroid effect was observed. As is often the case when a new experiment is added to a course, we had limited number of set-ups and technical problems with the use of older equipment. We plan to expand the number of set-ups so that we can give more students hands on experience with LTP measurements and increase the statistical power.

EVALUATION OF STUDENT WORK

We evaluated the work of the students in several ways. Each week each student turned in copies of his or her lab notebook pages, which were graded based primarily on format and completeness and averaged 4.83/5.00 points. For the first four project labs each lab group turned in a lab report that was used to incrementally build a literature review and writing skills. For the Ovariectomy lab, the students wrote a methods section and provided 15 pertinent references. For the Reproductive Behavior lab, the students wrote a methods section, a results section with graphs and statistical results and provided three to five new references. The first lab report from the Spatial Learning experiments included a method section and a brief review of the literature on spatial learning in rats with references, and the second lab report consisted of graphs of the results and statistical analysis of the data. The groups averaged 16.5/20 points on these reports, which were used as a starting point for the final journal style (*The Journal of Neuroscience*) report that each lab group submitted. The students were given detailed instructions for the final reports. The reports included 13 figures with statistical analysis and ranged from seven to ten pages of text and included from 14 to 27 references. The groups averaged 87.6/100 points with a range of 82 to 93 points. Many of the students had no prior experience with journal style reports and clearly benefited from working on and receiving feedback on the weekly reports. The quality of the different sections of the reports was variable making it clear that the students had subdivided the writing of the report. The depth of discussion was the weakest part of the reports. We plan to stress the literature review and discussion for future reports and have each member of the lab group take responsibility for the entire report.

As we have found that students have concerns about the impact of group work on their grades, we have each student complete a "Percent Contribution" form at the end of the semester in which she or he evaluates the contribution of each of the group members, including her- or himself, to the joint lab work. We use these scores to adjust the total points earned by each student for the lab.

ASSESSMENT

We had the students complete a Self Assessment of Learning Goals (SALG) instrument related to 23 specific goals for the course at the beginning and end of the course. Fig. 5 shows eight of the 13 goals in which the students reported significant improvement during that time.

Significant improvement was also seen in goals similar to *A-E* but in the students major(s) or from a disciplinary perspective. In addition, the students also indicated that the lab project had a strong impact on their achievement of the following goals:

1. Find scientific journal articles in one discipline using library/internet databases.
2. Find scientific articles from several disciplines using library/internet databases.
3. Obtain scientific data in a laboratory or field setting.
4. Work on a complex science problem with other students in my major(s).
5. Work on a complex science problem with other students not in my major(s).

We surveyed the students at the end of the semester to determine if they believed the learning objectives for the project (see **THE LAB PROJECT** section) had been accomplished. Of the 20 students who completed the survey, 90%, 95%, 100%, and 95% believed that objectives 1, 2, 3 and 5 had been accomplished respectively, while only 71% believed that objective 4 had been accomplished. For objective 4, several students noted that at this stage many of them do not have specific disciplinary strengths that they could bring to the project.

We also invited the students to comment on the project. Some of those comments are as follows:

1. I am a huge activist for animal rights and treatment (though not an extremist), so I thought I would become

very emotional in knowing that the rats had to be killed for our experiment. What I found, however, was that the procedure was mostly painless and cruelty-free for the animals, and the results of the labs helped in my understanding of neuroscience and research.

2. I learned I actually enjoyed working with the lab animals.
3. I learned that I enjoy surgery (though I'm not sure how good I am at it) and that I'm more interested in the behavioral and endocrinological aspects of neuroscience.
4. I learned to be more patient with the people in my lab group and how to divide work according to people's strengths.
5. I learned that science is not my best subject and how to professionally conduct scientific research.
6. I liked getting the "big picture" at the end by reflection on all of the procedures/experiments done to determine the effects of sex hormone treatment.
7. I learned that you don't have to be a professional to conduct sophisticated experiments and communicate them professionally. I learned self-confidence.
8. I learned that I can do things, and do them well, that I never thought I would do.
9. I was able to confirm my passion for the subject of neuroscience.

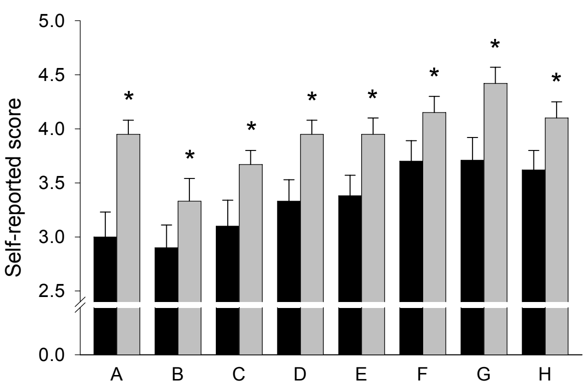

Figure 5. Self Assessment of Learning Gains (SALG) for the Introduction to Neuroscience Course for which significant improvement occurred. Mean pre-course (black) and post-course (gray) SALG scores are plotted above. Goals corresponding to letters were: *A*) Make an argument using scientific evidence from more than one discipline, *B)* Determine what is - and is not - valid scientific evidence in an area that is not my major, ***C*) Pose questions from more than one disciplinary perspective that can be addressed by collecting and evaluating scientific evidence, *D*) Learn about complex science problems that require insight from more than one discipline,** *E*) Communicate my research results to students not in my major(s), *F*) Understand the role of uncertainty in scientific data, ***G*) Extract main points from a scientific article and develop a coherent summary,** and *H*) Write reports using scientific data as evidence. * = Post-course SALG score was significantly different from pre-course SALG score at $p<0.05$. Students were also asked to rate the impact of the lab project on their SALG score for each goal. Those goals in which the students indicated the lab project had a strong impact are written in **bold**.

SUMMMARY AND CONCLUSIONS

In conclusion, the lab project we've described has enabled our students to meet the objectives for both the lab project itself and, more broadly, for the *Introduction to Neuroscience* course. By doing the lab project students increased their ability to conduct all phases of research from the literature review, to the lab bench, to the analysis of data, and finally to the communication of results. Another benefit of the project was that it provided students with a comprehensive examination of the relationship between the brain and behavior. Specifically, students gained a much richer understanding of how hormones can affect protein expression levels within different regions of the brain that lead to changes in neuronal function, general brain morphology and ultimately behavior. Based on the positive outcomes of our project we believe implementing similar lab projects in other science courses, irrespective of the research question or focus, will result in student gains in technical research skills, appreciation for the use of interdisciplinary approaches to answering complex scientific questions, and enthusiasm for the course.

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