ARTICLE
Characterizing Mystery Cell Lines: Student-driven Research Projects in an Undergraduate Neuroscience Laboratory Course

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Inquiry-based projects promote discovery and retention of key concepts, increase student engagement, and stimulate interest in research. Described here are a series of lab exercises within an undergraduate upper level neuroscience course that train students to design, execute and analyze their own hypothesis-driven research project. Prior to developing their own projects, students learn several research techniques including aseptic cell culture, cell line maintenance, immunocytochemistry and fluorescent microscopy. Working in groups, students choose how to use these techniques to characterize and identify a "mystery" cell line. Each lab group is given a unique cell line with either a neural, astrocyte, or Schwann cell origin. Working together, students plan and execute experiments to determine the cellular origin and other unique characteristics of their mystery cell line. Students generate testable hypotheses, design interpretable experiments, generate and analyze data, and report their findings in both oral and written formats. Students receive instructor and peer feedback throughout the entire project. In summary, these labs train students the *process* of scientific research. This series of lab exercises received very strong positive feedback from the students. Reflections on student feedback and plans for future improvements are discussed.

Key words: cell biology, molecular biology, cell culture, immunocytochemistry, hypothesis-driven research, open-ended project, inquiry-based instruction, student-driven research project, research design, upper level neuroscience course, neuroscience education

Benefits of investigative and open-ended laboratories for undergraduates (Sundberg and Moncada, 1994) are numerous. Studies suggest that student retention of key concepts and critical thinking skills are enhanced by inquiry- and project-based learning strategies (Council, 2003; Rissing and Cogan, 2009; Treacy et al., 2011). Open ended projects, such as the student-driven research projects described here, can increase student motivation and engagement (Wiegant et al., 2011). Project-based exercises have been shown to promote a stronger student interest in science research (Treacy et al., 2011). Many reports describe inquiry-based learning approaches in neuroscience and neuroscience-related courses (Goyette and DeLuca, 2007; Birkett, 2009; Chase and Barney, 2009).

Here we describe a lab design that prepares and then challenges students to generate their own hypothesis-driven set of experiments to identify a cell line using cellular and molecular techniques. These labs guide students through all aspects of the *process* of science research. This lab design is ideally suited for undergraduates to experience all aspects of science research in an upper level neuroscience or a cell biology course. The goals of the student-driven independent research project are to:

1) engage and challenge students in the interdisciplinary field of neuroscience
2) enhance students' ability to generate testable hypotheses
3) improve students' ability to generate an experimental rationale and experimental design
4) have students work as a team to execute these experiments
5) enable students to collect and critically analyze their data
6) further train students how to clearly present their data as a lab report and an oral presentation
7) enable the research project to fit within a 8-week time constraint during a semester.

THE COURSE

The Principles of Neuroscience class is an upper level course that is required for a neuroscience concentration for biology majors and can serve as an elective for science majors. In the near future, this course will also be a requirement for the newly proposed cognitive and brain concentration for psychology majors. This Principles in Neuroscience course is taught at a small, predominantly undergraduate liberal arts college, Assumption College. Thus far, the course has been taught twice, and both times it has had a majority of biology majors with a few psychology majors. Before taking Principles of Neuroscience, all students must take at least two core biology courses: Concepts in Biology and Genetics. In addition, biology majors must take at least one of two upper level biology classes: Cellular and Molecular Biology or General Physiology. Psychology majors must take an upper level Physiological Psychology course as a prerequisite. Therefore, all students have a strong foundation of key biological concepts and have practiced fundamental lab skills. Some biology majors have more extensive background in cellular biology or physiology, while psychology majors have a background in neurophysiology and a unique perspective on brain

function. Students work in groups of three or four in the Principles of Neuroscience course. The instructor carefully selects the groups such that combinations of strengths (cell and molecular, physiology, psychology) are represented in each group. Students are made aware of the rationale of the group selection and are encouraged to capitalize on having an "expert" in one of each of these fields.

For the project described here, all students will learn and perform a variety of techniques including aseptic cell culture, cell line maintenance, immunocytochemistry, fluorescent microscopy, digital photography and basic statistics. Students who have taken Cell and Molecular Biology will utilize their previous training in cell culture and immunocytochemistry. These students serve as the "experts" within their group for these techniques. All students will also utilize and build upon microscopy skills and understanding of statistics previously taught in Concepts in Biology and Genetics.

The entire course runs for 15 weeks. A single lab section is scheduled for three hours and is limited to 16 students. The lab is divided into three units over the semester: 1) neuroanatomy, 2) neurophysiology and 3) cellular and molecular neurobiology. These second and third units both have student-oriented independent projects. However, the focus of this paper is to describe the planning and execution of the student-driven independent project during the third unit of the lab that focuses on molecular and cellular neurobiology. These lab exercises are appropriate for other undergraduate courses in neuroscience or cellular and molecular biology.

STUDENT PREPARATION FOR INDEPENDENT RESEARCH PROJECT

Technique labs

Prior to the start of the independent "mystery" cell research projects, students complete three "technique" labs, as done by Switzer and Shriner (2000). These three technique labs train students to: 1) successfully maintain their mystery cell line using aseptic cell culture techniques, 2) count their cells with a hemacytometer, 3) perform immunocyto-chemistry (ICC) and 4) use fluorescent microscopy and digital imaging to capture images of their cells. The techniques learned in these labs are required for the independent projects in which students are charged with identifying and characterizing their "mystery" cell line.

In technique lab #1, students are introduced to aseptic cell culture and cell line maintenance. The instructor reviews and provides a cell culture handout, which describes steps involved in cell maintenance. The instructor also models how to feed and divide cells in the cell culture hood. Each student is given their own T25 flask with their "mystery" cells and is charged with maintaining his/her cells throughout the remainder of the semester. Each student within a single group receives the same "mystery" cell line. Each group within a lab section has a distinct "mystery" cell line.

During technique lab #1, students also learn how to use a hemacytometer to calculate cell density. Students use this information to determine the optimal plating density of their cells. The optimal plating density is used to keep cells healthy and to keep the timely demands of cell feeding at a minimum. Prior to technique lab #1, the class has discussed at least one primary science article that utilizes cell culture technique to help students become more familiar with this technique. The values and limitations of cell culture are discussed in lecture and lab before students are charged to take care of their own cell line.

Students learn that "real" research does not easily fit into the once-a-week, three-hour time slot. Students realize that in order to keep their cells alive, they must come to lab outside of the regularly scheduled time to feed and split their cells. We have been pleasantly surprised by how well all students rise to this challenge and successfully maintain their cells. We have not yet had any students who have failed to maintain their cells. At Assumption College, we are able to keep the doors to the cell culture lab closed but unlocked between 7:30AM and 6:00PM five days a week. A sign-up sheet with time slots for both aseptic hoods is provided on the exterior of the door. This sign-up sheet helps avoid long lines of students waiting to use the hood and it blocks out times during which the Cell and Molecular Biology lab is running in the room. The latter is important because it prevents students from walking into the cell culture area while it is in use by another lab course.

During technique lab #2, students learn ICC. In order to perform ICC, students must plate some (but not all) of their mystery cells on glass coverslips on the day before the regularly scheduled lab. Despite the amount of time students must come to lab outside of the scheduled lab time, students appear enthusiastic. Students can work in groups and divide the work amongst their group members.

During technique lab #2, students perform ICC. Students fix their "mystery" cells that have grown on glass coverslips with paraformaldehyde. Students subsequently rinse and block their cells. Cells are then incubated with a primary antibody solution consisting of monoclonal tubulin antibodies and polyclonal actin antibodies diluted in block. These two antigens are present in all of the cell lines. This is important because: 1) students have positive immunostaining for their first ICC run and 2) staining for actin and tubulin does not reveal the identity of the "mystery" cell lines (Table 1). Students then rinse the cells, apply a secondary antibody solution and rinse again. Students mount the glass coverslips with their cells on glass microscope slides. Please see the materials and methods section for more detail. During the next lab session, students will visualize their stained cells on a fluorescent microscope.

During lecture and prior to the ICC lab, the class has reviewed three primary science articles in detail. At least two of these primary science articles employ ICC, cell culture and statistics. Thus, we have discussed ICC prior to the start of ICC in lab. In addition, we cover the ICC procedure in great detail and refer to the lab manual during lecture. We review the biological mechanism of ICC. This enables students to understand the capability of ICC and the rationale of the ICC procedure. This is critical when students will undoubtedly need to trouble-shoot during the

independent project phase.

During technique lab #3, students learn to use the fluorescent microscope and attached digital camera. The instructor meets with each lab group separately for approximately 30-45 minutes. During this time, the instructor teaches the students how to use the microscope and capture images of cells. Directions for the fluorescent microscope are also written in detail in the course lab manual. Each group is given a quiz at the end of the training session. The quiz challenges the group to work together to view and take a picture from a slide provided by the instructor. The instructor observes the group to ensure they are competent on the microscope before the group is permitted to leave.

After technique lab #3, each group is charged with returning to the microscope outside of the scheduled lab time to capture images of their cells. Images must show staining of both antigens (actin and tubulin) separately and as an overlay. Students must make a figure and figure caption for their images. Their figure is submitted to the instructor for a grade at the beginning of the next scheduled lab. This assignment helps students practice using the microscope and how to illustrate future data generated by their independent projects.

Cell line	Cell Type	Antigens expressed
D1 TNC1	Astrocyte	GFAP, actin, tubulin
RSC96 and RT4-D6P2T	Schwann cell	MAG, s100, actin, tubulin
HCN-1A and Neuro2A	Neuron	Neurofilament protein, actin, tubulin

Table 1. Select antigens expressed by distinct cell lines

Students create an experimental plan and timeline

After students have learned these basic techniques, lab groups are challenged to design a series of experiments to identify and characterize their "mystery" cell line. Students are told that their cells have either an astrocyte, neural, or Schwann cell origin. Students are to imagine they are the first ones to discover their "mystery" cell line and will submit a publication that characterizes this mystery cell line. The instructor gives a few examples of possible questions to help initiate group discussion:

1) Does their cell line express cell specific antigens? For example, do their cells express neural specific antigens such as neurofilament protein? Do their cells express the astrocyte-specific antigen, glial fibrillary acidic protein (GFAP)?
2) How do their cells react to injury? Do the cells swell or die after an *in vitro* scrape injury? Does the antigen expression change after injury?
3) What is the morphology of their cells? Do their cells appear different if they are plated on extracellular matrix molecules compared to glass?
4) Can you apply findings from primary science articles discussed in class to pose other questions about your "mystery" cell line?

After the discussion has begun, the instructor provides each student with a packet of specification sheets for numerous reagents that are available to them. Examples include primary antibodies (against GFAP, neurofilament, MAG, etc…), secondary antibodies (goat anti-mouse conjugated to ALEXA 488 and goat anti-rabbit conjugated to ALEXA 568.), vital cell stains (propidium iodide) and nuclear stains (DAPI). This gives the opportunity for students to learn how to read a specification sheet. This is particularly important when choosing the appropriate primary and secondary antibody combination, determining the correct working concentration of an antibody or when trying to learn how to use a reagent they are not yet familiar with (such as propidium iodide.) Students can use the instructions on a specification sheet as well as the references cited therein to determine how to use a reagent like propidium iodide that we've discussed in class but have not yet used in lab. The location and aliquot size of each reagent is written on each specification sheet. Students are not absolutely required to limit their experimental reagents to this list but it is highly encouraged.

Groups are charged with generating questions, then hypotheses, then designing experiments. A group of four students must generate five questions; a group of three must generate four questions and so forth. After each group has selected their questions, they must then generate testable hypotheses. Students must plan a detailed experimental design that tests each hypothesis. The experimental design must include positive and negative controls. The experimental design must specifically include which primary and secondary antibodies are used for a given experiment. Students must learn how to successfully match a primary antibody with an appropriate secondary antibody (for example, pairing a monoclonal primary antibody with a goat anti-mouse secondary antibody and not a goat anti-rabbit secondary antibody). Students must also list what concentration they will use for each antibody.

After the in-class discussion, the instructor provides a handout that guides the students through the question, hypothesis, and experimental plan sequence. Students are also asked to predict possible outcomes and the interpretation of these outcomes. The instructor models this process with a few examples during class. The instructor also points out the strong similarities between this class assignment to grants that researchers must write for funding.

During the next lecture, each group presents their own questions, hypotheses, experimental plan, possible outcomes and interpretations as an oral presentation prior to the start of the designated independent research labs. Immediately after each presentation, students in the audience provide anonymous feedback that describes at least two strengths and two weaknesses of the presenting group's experimental design. Students in the audience write their constructive criticisms on index cards that are collected after each presentation. These cards are read by the instructor and shared to the class prior to the next group's presentation. The instructor's own comments are also included in this immediate feedback process. In this way, potential experimental flaws are addressed and corrected before experiments in lab begin. Each student in

the audience becomes an active participant and reviewer by supplying anonymous feedback. We have found this to be particularly useful and students experience a taste of the anonymous peer review process. The process of grant reviews is also covered during class at this time. Students realize their assignment shares some similarities to what researchers must do to obtain research funding.

In order to ensure the experiments can be completed in a timely fashion, students generate a time line for their research projects. Each group must submit a detailed set of plans of which procedures and experiments are done each day and which group member will perform each protocol. For example, each group must detail who will feed and maintain their cells on which days, prepare the cells for ICC, conduct ICC, analyze and capture data of each experiment, etc…. The instructor emphasizes the importance of planning additional time for unexpected problems with experiments. The document detailing the time line and work assignments is signed by each member of the group to legitimize the plan. This signed document is submitted to the instructor for a grade.

THE INDEPENDENT PROJECT EXECUTION

Students have four weeks to characterize their mystery cell line. Students follow the experimental plan and time line they generated, as described above. All students are required to be present at the beginning of each traditional three-hour lab session during the independent project execution phase. This provides the opportunity for general lab announcements and more importantly, enables the instructor to gauge the progress of students' research. Each week in lab, the instructor meets with all groups to learn of their successes, frustrations and difficulties. The instructor provides suggestions when inevitable challenges arise. These student-instructor discussions help the students learn how to trouble-shoot and critically think about their experiments.

Towards the end of the independent project phase, students work together in their assigned groups to generate a PowerPoint presentation that describes their experiments. After the four weeks of independent research, each group gives a 15-20 minute presentation during the regularly scheduled lab. The instructor spends time in lecture giving examples of an effective PowerPoint presentation. The students are also referred to a book entitled, "A Student Handbook for Writing in Biology" (Knisely, 2009). This book was required for the students to purchase in their prerequisite courses. It is an excellent resource for preparing a PowerPoint presentation. Students are also encouraged to use the "Oral Presentation Grading Rubric" (Supplemental Figure 1) while preparing their presentation. All grading rubrics for the semester are in the student lab manual. The students are told that this grading rubric is used by the instructor to evaluate their oral presentation. Thus, there are no surprises in regard to the components of the oral presentation that will be evaluated by the instructor.

Approximately one week after student oral presentations, each group submits a written lab report describing their independent research projects. The one-week delay between oral presentation and lab report allows students to incorporate instructor's comments from the oral presentation into their written reports. Students are encouraged to consult Knisely (2009) as well as the "Lab Report Grading Rubric" (Supplemental Figure 2) while preparing their lab report.

Each member of the group must sign the final copy of the lab report before giving it to the instructor. The signatures confirm that each signee contributed to the lab report and deserves the grade that is earned by the lab report.

After submission of the group lab report, students provide an assessment of each of their group members. Students score all members of their group, including themselves, using a 1-100 scale that reflects each member's contribution to the project, their ability to come to meetings prepared and their ability to work together as a team. Students are informed of this peer assessment opportunity and the possible repercussions of the peer assessment at the beginning of the project. Students know that only the instructor will see the peer evaluations. If a strong trend amongst a few students is seen, this trend will be reflected in the final grade of the lab report and oral presentation for a particular student. The student is privately informed of the instructor's decision and allowed the opportunity to discuss this further with the instructor, if requested.

MATERIALS AND METHODS

Antibodies

Primary antibodies used were monoclonal tubulin antibody (T5168, Sigma), polyclonal actin (AB1801, abcam), monoclonal neurofilament antibody (MAB1623, Millipore), polyclonal MAG antibody (34-6200, Invitrogen), monoclonal S-100 antibody (clone EP1576Y, Millipore) and polyclonal GFAP antibody (AB5804, Millipore). Secondary antibodies used were goat anti-mouse ALEXA 488 and goat anti-rabbit ALEXA 568 from Invitrogen.

Cell lines and culture media

Cell lines were obtained from ATCC. Cell lines were: D1 TNC1 (CRL-2005, astrocytic cell line), RSC96, (CRL-2765, a Schwann cell line), HCN-1A (CRL-10442, a neuronal-like cell line), neuro2A (CCL-131, a neuronal-like cell line.), RT4-D6P2T (CRL-2768, a cell line with Schwann cell properties). A list of these cell lines with project-relevant antigen expression for this project is found in Table 1.

All cell lines were maintained in DMEM (Lonza) with 10% fetal bovine serum (Gibco) and 100,000 units of penicillin and 100 mg of streptomycin (Gibco) per 500ml of media. To transfer the cells in a T25 flask to another sterile dish, cells were rinsed with non-serum containing DMEM and treated with 1ml of 0.25% trypsin (Gibco) for 5 minutes in a 37^0C incubator. After cells were no longer adhered, 4mls the DMEM with 10% FBS were added to the T25 flask to inactivate the trypsin. The cell density was calculated using a hemacytometer and plated at a known density (usually 40,000-60,000 cells/per ml) into a new T25 flask and/or into a 12 well plate with sterile glass coverslips

(13mm in dm, Electron Microscopy Science, 63780-01) placed in the bottom of each well. A T25 flask had a total of 5 mls media and a single well of a 12 well plate had a total of 2 mls of media.

Students used one of two cell culture hoods (Sterilgard Hood Class II Type A/B3), an inverted stage microscope (Olympus CK2) and a $C0_2$ incubator (Labline 490) to maintain their cells.

Other reagents

Propidium iodide (P3566, Invitrogen) was used as a vital cell stain. DAPI (D1306, Invitrogen) was used as a nuclear stain. Students followed the protocol listed in the specification sheets of these reagents. Some students chose to study their cells plated on laminin (23017-015, Invitrogen) coated coverslips rather than glass. In this instance, students coat their coverslips with 20μg/ml of mouse laminin-1 for one hour at room temperature as described previously (Lemons and Condic, 2006).

Immunocytochemistry (ICC*)*

Prior to ICC, cells were plated on sterile glass coverslips placed into the bottom of wells in a 12 well plate. After cells were sufficiently adhered, they were fixed for 10 minutes with a warmed solution containing 4% paraformaldehyde and 30% sucrose in PBS. Cells were rinsed for five minutes with 2mls of room temperature PBS per well. This was repeated for a total of three rinses. One ml of blocking solution was placed into each well and allowed to incubate for 30 minutes at room temperature. Blocking solution consisted of 5% normal goat serum and 0.1% Triton-X in PBS. Primary antibodies were diluted in block solution and 500μl of primary antibody solution was placed into each well and allowed to incubate 30 minutes at room temperature. Cells in each well were rinsed with 2 mls of PBS for five minutes. This step was repeated two more times. Secondary antibodies were diluted into block solution at 1:1000 and filtered through a 0.45 micron filter. The filtered secondary antibody solution was placed on cells and allowed to incubate for 30 minutes at room temperature in the dark. One half ml of filtered secondary antibody solution was used per well. Following the secondary antibody incubation, cells were rinsed with PBS as described earlier. Coverslips were then mounted onto -glass microscope slides with Fluoromount (Southern Biotech.) Microscope slides were placed into horizontal slide holders and kept at 4^0C overnight. The cells could be analyzed as early as the next day. When slides were not on the microscope, they were kept in slide boxes at 4^0C for the duration of the course.

Students were informed that ICC could run in one long day or in two short days. The procedure for a two-day procedure of ICC is very similar to the protocol described above. The one considerable difference is that the primary antibody solution incubates overnight at 4^0C instead of 30 minutes at room temperature. After this overnight incubation step, cells were treated in the same way as described above, beginning with three rinses with PBS and then proceeding with a secondary antibody incubation.

Microscopy

Students are trained to use one of two fluorescent microscopes with attached cameras: an Olympus BH2 fluorescent microscope and an Olympus cooled RTV camera or a 90i Nikon fluorescent microscope with an Andor Clara-E camera. Both microscopes and cameras are connected to different PC computers equipped with software that communicates to the camera.

***In vitro* cell injury model**

In class, we had discussed research models of CNS injury including the *in vitro* mechanical cell scrape technique (Kornyei et al., 2000; Zhu et al., 2007). Some students chose to use this model to characterize their mystery cell line response to injury. Cells grown to confluency were injured by making scrapes along the bottom of the dish with a sterile pipette.

RESULTS

Each group has a different cell line, and therefore, the results from experiments from a given lab section are always unique. Possible cell line origins are astrocyte, neural, and Schwann cell. Most groups initially use ICC to determine if their mystery cell line has cell-specific antigens present. For example, a group may find their mystery cell line to be negative for GFAP and MAG and positive for neurofilament. In this instance, the group would conclude that their cell line has a neural-like origin. Students learn to become efficient by simultaneously testing for the presence of two antigens in one well. Students can use two antibodies during an ICC run (with one monoclonal antibody and one polyclonal antibody.) Students also use this option to include an internal positive control. For example, while testing for the presence of GFAP using a polyclonal GFAP antibody, students will also use a monoclonal actin antibody in the same well. Students have previously confirmed the presence of actin in their cell line and this serves as an internal positive control to confirm ICC and the fluorescent microscope are working properly. Students are highly encouraged to include a negative control, such as the absence of a primary antibody.

After this initial characterization, students become very creative in their approach to characterizing their cell lines. For example, one group studied the effect of distinct nerve growth factors on the vitality and growth rate of their mystery cells. They determined if their cell line was NGF or NT3 dependent. Another group studied the effects of mechanical scrape injury on cell vitality, cell morphology and expression of cell-specific markers. The possibilities are numerous and the students seem enthusiastic to ask and answer questions about their mystery cell line.

DISCUSSION

Although we do not have quantitative data to compare this independent project approach to more traditional technique-driven labs in other undergraduate neuroscience courses, our impression is that students appear to genuinely enjoy planning, executing and analyzing their own research project. In anonymous course evaluations,

students have commented on how great it is to conduct an experiment that "…even the instructor does not know how it will turn out." Students have said that learning techniques for the purpose of using the techniques later for a project of their own design helps motivate them in lab. One student said, 'Knowing I would have to use these techniques again on my own helped me to pay closer attention in lab." Students said that while the research project was sometimes frustrating, it was also very rewarding when it finally worked. We feel that this sentiment accurately reflects the feelings often associated with novel scientific research. It suggests that students are indeed experiencing some genuine aspects of science research. In fact one student told the instructor that she enjoyed the research project so much that she could now understand why her brother was enrolled in graduate school in the sciences. In addition this student now plans to enroll in graduate school.

While most students enjoyed planning, conducting and analyzing their own research projects, one student expressed frustration by the "loss of time in lab" dedicated to independent research projects. This student expressed a desire to spend more time learning additional research techniques in lieu of conducting independent research projects with techniques learned previously. This is a continual struggle for many instructors. However, we feel that quality is more important than quantity. These independent projects enable students to learn about the *process* of science research and goes well beyond mastering techniques. These independent projects help students learn that techniques are tools used to answer questions and coming up with answerable questions is a key component of good experiments. We feel that the rich lessons learned from these projects outweigh benefits of students learning how to merely perform techniques. In the future, the instructor will make this point more clearly to the students.

Previous studies provide support of benefits gained by employing independent research projects within the context of a lab course. For example, in a project-oriented undergraduate neuroscience course at Hope College, students reported a significant improvement in 13 of the 23 goals for the course in a self-assessment of learning gains (Chase and Barney, 2009.) These goals included extracting main points from a scientific article and developing a coherent summary, understanding the role of uncertainty in scientific data and making an argument using scientific evidence from more than one discipline. In a genetics course with a hypothesis-driven research project using *Saccharomyces cerevisiae*, there was a significant increase in the post-lab student self-assessment compared to the pre-lab student self assessment. Students reported a greater understanding of mutagenesis, sterile technique, and positive and negative controls (Marshall, 2007.)

We plan to enhance the independent project experience in three ways. First, we will incorporate a peer-review process for lab reports. Students will be required to submit their lab reports to their peers for peer evaluation prior to their final submission to the instructor. Each student will be responsible for providing constructive criticism of one portion (introduction, methods, etc.) of a lab report. Students must describe at least three strengths of the report and three areas in need of improvement. The peer evaluators need to also suggest methods of improvement. These comments from all sections of a single manuscript will be collected and returned to the authors anonymously through the instructor in a timely manner. The peer review comments will also be submitted to the instructor for evaluation. This peer review assignment provides several benefits. Students will experience the peer review process of science research and will practice critical thinking skills by judiciously reading another group's manuscript in progress. Students will also be exposed to other ways of presenting and interpreting data by reading other students' lab reports. Authors will have the opportunity to edit their manuscript prior to final submission.

Secondly, the instructor will invite colleagues in the Natural Science Department and Psychology Department to attend the final student oral presentations during lab. Students will be highly motivated to prepare an impressive presentation to a larger, knowledgeable audience that will likely ask questions. Colleagues will be more informed about the nature of the research projects that are required in this course.

Thirdly, the instructor will schedule time to meet with each group shortly after their project plan presentation and before their project plan execution. During this meeting, the instructor will explain and suggest basic statistical analyses that may help strengthen each group's project. All students have been previously exposed to basic concepts about statistics through the required courses of Concepts in Biology and Genetics. In addition, statistics has been discussed during lecture of this neuroscience course during critical analysis primary science articles. However, this instructor-group meeting will provide the additional time needed to discuss preferred statistical tests for each group's project design.

This independent project can be easily modified to better suit the needs of a particular instructor or course in several ways. For example, students can be required to share their research findings as a poster presentation rather than an oral presentation. The poster presentation could be open to the entire Department and other instructors could serve as judges and evaluate each group's ability to design and present their poster. This project can also be modified for courses other than neurobiology by selecting cell lines that may be more appropriate for another course topic. For example, in a cancer biology class, cell lines derived from breast or colon cancer could be used. Similarly, cell lines from the immune system could be used for an immunology course.

SUMMARY

This paper presents a cellular based student-generated research project that is done during the semester within an upper level undergraduate neuroscience course. The paper includes steps to prepare students for a hypothesis driven science research project. Preparation involves the use of "technique labs" and training students how to design

and plan a series of interpretable experiments. Feedback from students and reflections for improvement are also discussed. In sum, student-designed hypothesis-driven research can be successfully conducted during the semester within an undergraduate course. This form of inquiry-based learning can enhance student understanding and interest.

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Received August, 01, 2011; revised October 06, 2011; accepted November 21, 2011.

This work was supported by the NIH through an AREA R15 grant awarded to Dr. Michele Lemons. The author thanks Dr. Kimberly Schandel and Dr. Owen Sholes for careful reading of this manuscript. The author is grateful to Dr. David Crowley who shared grading rubrics.

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Supplemental Figure 1

Grading Rubric for Oral Presentations					
Organization and Content (75% total)	**Consult Chapters. 8 and 4 in Knisely Writing Manual**	**10 (Outstanding) 9(Very good)**	**8 (Good)**	**7-6 (Fair)**	**5-0 (Poor)**
15%	**Introduction** - Capture audience interest, clearly state objectives, give relevant background information	Clearly presents the objectives and rationale of the experiment, along with background material that is appropriate	Only partially presents the objectives and/or rationale of the experiment OR fails to capture audience interest due to inadequate or irrelevant background information	Limited information presented and objectives and/or rationale of the experiment poorly presented	Inadequate or no background
15%	**Body – Methods** Describe methods in sufficient detail for audience to understand how experiment was carried out, avoids too much text and uses pictures/diagrams instead	All variables are properly identified and explained. The experimental design is adequate. This includes: data to be collected, controls, techniques utilized, number of replicates. Detail appropriate for oral presentation	Only some of the variables and design issues are identified and explained. Errors in design, inappropriate use of techniques, etc. OR too much detail for oral presentation	The variables and design issues are not properly identified nor explained OR only a list of methods and/or materials presented	No Methods included in body of talk
25%	**Body - Results** Graphical or tabular presentation of data	Complete and adequate. Tabular and/or graphic representations of data are appropriately used and formatted for effective presentation	Data presentation not adequate e.g., figures improperly presented or formatted (e.g., captions, titles, axes labels, etc.) OR data not presented in clear manner for oral presentation	Essential data not included in presentation OR data is inadequately presented in many places; inappropriate data presentation	No Results presented
20%	**Closing-** Summarize results, give possible explanations for results, compare results to those in the literature, point out errors or inconsistencies, discuss possible future research and implications for or applications to daily life	Appropriate interpretation and discussion of the outcomes of the experiment. Possible implications/further experiments are proposed	An adequate interpretation of the data is presented, but it is not related to possible implications or additional experiments are not suggested OR vice versa	The data and implications are not adequately discussed	No closing presented
Delivery (25% total)	**Consult Chapter 8 in Knisely**				
10%	**Delivery** Speaker established rapport with audience, style, mannerisms, and appearance did not detract from content of presentation	Poised, good eye contact, no distracting gestures or mannerisms, loud enough, not too fast or slow, spoke clearly, stayed within time limit, used appropriate scientific language, did not read speech, did not turn back on audience when using visual aids, fielded questions with poise	1-2 errors in these categories	2-4 errors in these categories	>5 errors in these categories
10%	**Visual Aids** Clearly displayed appropriate amount of information, complement spoken words	Simple, legible, organized logically, important findings stand out	Some visual aids distracting OR not organized OR contain too much information; illegible	Most visual aids distracting OR not organized OR contain too much information; illegible	Visual aids poorly designed; show little effort
5%	**Teamwork** Each member contributed effectively to the presentation	Well-coordinated, smooth transitions from speaker to speaker, equal division of labor, non-speaking partners attentive and not distracting	Some minor inconsistencies in style or tone evident	Requires significant revisions, rehearsal, and coordination	Shows no evidence of rehearsal and/or coordination

Supplemental Figure 2

Grading Rubric for Lab Reports					
		10 (Outstanding) 9 (Very good)	**8 (Good)**	**7-6 (Fair)**	**5-0 (Poor)**
Format and Style (10%)					
5%	**Appearance, Appropriate Writing Conventions, Overall Style**	No grammar or spelling errors. Past tense and passive voice used throughout. Proper scientific notation and SI units and symbols used. Concise, clear, consistent tone and style, with signs of revision.	1-2 errors in formatting, tense, grammar, spelling, word or number usage, some minor inconsistencies in tone or style, a few sections not clear or concise	2-4 errors in formatting, many errors in tense, grammar, spelling, word or number usage, serious inconsistencies in tone and/or style, extensive revision needed	>4 errors in formatting, grammar, etc., disregard for proper writing conventions, no evidence of proof-reading
5%	**References**, use the Name-Year System, see Knisely handbook	Appropriate number and quality of citations formatted using N-Y system. Used properly.	Appropriate references used but not formatted properly OR formatted correctly but not used properly	Inadequate or inappropriate references used, may or may not be cited properly	Inadequate or no references used, cited incorrectly
Content (90%)					
5%	**Abstract**, summary of the entire report in <250 words,	Abstract summarizes ALL sections of report concisely, states key findings	Abstract summarizes all sections of report but is not concise or is too brief or contains experimental details	Abstract does not summarize all sections of report or contains excessive experimental detail	Abstract fails to summarize report and is of inappropriate length
15%	**Introduction**, presents background information necessary for results, purpose of experiment stated, justification of methods included	Clearly presents the objective and rationale of the experiment, along with appropriate background material, explains what is currently known and what is not, states goals of experiments, states rationale for methods	Only partially presents the rationale for the experiment and methods OR background information is inadequate or irrelevant , goal of experiments not clear	Limited or no background information presented and basis and rationale for the experiment and methods poorly presented	No background presented and/or no rationale for experiment and methods
10%	**Materials and Methods**, brief description of procedure with reference to lab manual, changes noted, detail sufficient to repeat experiment	All parts of experiment described in manner that allows replication, changes noted, how data was collected and analyzed is clearly stated	Only some of the parts are mentioned OR changes are not noted OR too much detail is given.	Few parts are explained, changes are not noted OR detail is excessive	Lab manual is cited by no methods are described OR lab manual is rewritten
15%	**Results as figures and tables**, figures with informative captions, tables with appropriate titles	Tabular and/or graphic representation of data are appropriately used and formatted, graphs are labeled appropriately, tables have titles, figures have captions	Data presentation not adequate, OR tables and graphs are not entirely correct (lack appropriate labeling, titles, captions, etc.)	Tables and graphs are poorly constructed (more than 2 errors), one or more tables or graphs is missing	Multiple errors and/or some data missing
15%	**Results written as text** written description of data is logical and well-written, refers to figures and tables	Written description of data is complete, concise, easy to follow	Text does not summarize data fully, text has several errors	Text poorly describes data	No text describing data, some data is missing
30%	**Discussion,** results stated, justified and put in context of existing information, any errors stated and discussed	Appropriate interpretation and discussion of the outcomes are well written, significance explained, any errors or discrepancies discussed	An adequate interpretation of the data is presented, but it is not put in context OR vice versa, impact of errors not discussed	The data and implications are not adequately discussed, justification is not clear	Improper conclusions drawn OR conclusions not justified, errors not addressed

***Zero points will be awarded for a section that is omitted.