ARTICLE
Chick Embryonic Primary Astrocyte Cultures Provide an Effective and Scalable Model for Authentic Research in a Laboratory Class

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Cell culture provides an impactful tool for undergraduates to study a range of neurobiological processes. While immortalized or cancer cell lines offer a level of convenience for undergraduate research, particularly for larger scale course-based undergraduate research experiences (CUREs) or project-based learning (PBL), primary cell cultures more closely retain the characteristics of the tissue of origin, allowing students to engage in a wider range of authentic research projects. Astrocytes have gained increasing attention for their role in modulating neuronal viability and are at the forefront of neuroprotection research. Here we describe a method of primary astrocyte culture preparation, derived from embryonic day 8 chicken embryos, optimized for a cell biology laboratory class. The primary astrocytes, prepared and maintained by undergraduates, were used as the model system for student-centered research projects in which students investigated cytoskeletal changes in response to drug treatments. Students reported several learning gains from the experience. The ease of the primary culture method for novice research students allows greater flexibility in designing authentic and scalable research experiences.

Key words: primary cell culture; astrocytes; actin; chick embryo; course-based undergraduate research experience (CURE), project-based learning (PBL)

Course-level research experiences provide large groups of students the opportunity to engage in authentic scientific practices. Course-based undergraduate research (CUREs) and project-based learning (PBL) are two models for providing impactful experiential learning in laboratory courses. CUREs immerse students in authentic research through iterative scientific experimentation with the intent of producing results that are novel and/or of interest to the greater scientific community (Corwin et al., 2015). PBL shares similarities with CUREs, but places more emphasis on student choice in the research process and producing a public product (e.g., presentation outside of the college or university; Wallace and Webb, 2016). With PBL, the scientific process should be authentic (i.e., addressing an important research question using the scientific method), but the results do not necessarily need to be novel and appropriate for peer-reviewed publication.

Providing large groups of students the opportunity to engage in authentic research is an inclusive teaching practice. Students' barriers to participating in extracurricular research, such as work commitments or apprehension towards faculty, are removed when authentic research is scaled up and embedded within the required curriculum (Bangera and Brownell, 2014). A key element to the success of CUREs or PBL is the scalability of the research project and ability for undergraduates to master the scientific technique within a relatively short time-frame.

Cell culture is an impactful model for studying a range of cellular mechanisms and has been instrumental in the development and screening of small molecule therapeutics (Jaroch et al., 2018). The ease and practicality of obtaining cancer or immortalized cell lines from a frozen stock, makes this an attractive model for course-based research. Cell lines, however, do not typically retain the characteristics of native tissue, diminishing their appropriateness for a range of neurobiological studies (Lorsch et al., 2014). In contrast, primary cells are taken directly from the target tissue and often utilized without passaging, thus better retaining the primary characteristics of the tissue (Kimelberg HK, 1983).

Primary cells require additional time and greater technical expertise (e.g., tissue dissection) compared to cell lines, creating challenges for their use in CUREs or PBL. This is particularly true for primary neuronal cultures which, due to the timing of neurogenesis, ideally require dissection of younger embryos with delicate embryonic brain tissue, performed in a swift manner to ensure viability. Astrocytes, on the other hand, are more resilient. High quality primary astrocyte cultures are less technically challenging to prepare than neurons because they are typically derived from an older developmental stage and better withstand the slow dissection technique of novice researchers.

Astrocytes are a compelling research topic for undergraduates, as they play a central role in neurodegenerative disease (Garden and Spada, 2012). Over the last few decades, the failure of clinical trials aimed at combatting amyotrophic lateral sclerosis, Alzheimer disease and Parkinson disease, have led neuroscientists to pay closer attention to non-cell autonomous mechanisms of neuronal cell death (Hamby and Sofroniew, 2010). Of particular therapeutic interest is the dual role of astrocytes in both combatting and contributing to neuronal death (Sofroniew and Vinters, 2010). Pro-survival mechanisms include astrocyte-dependent antioxidant support of neurons (Haskew-Layton et al., 2010; Haskew-Layton et al., 2013), while pro-death mechanisms include the release of damaging pro-inflammatory molecules from astrocytes with a reactive hypertrophic morphology (Sofroniew and Vinters, 2010).

We describe here a scalable technique to generate primary astrocytes in a cell biology laboratory class of 14-18

students, in which each student performs an individual dissection of an embryonic day 8 chicken embryo optic tectum, followed by tissue dissociation with small groups of students. Upon subsequent confluency of the cultures, students designed and performed experiments to monitor changes in the actin cytoskeletal in response to drug treatment (using a drug of their choice). Reorganization of actin is integral to the transition of astrocytes from a normal to a reactive morphology (Lee et al., 2018; John et al., 2004), highlighting the scientific relevancy for undergraduate students to investigate astrocytic actin.

MATERIALS AND METHODS

Pedagogical study design

The pedagogical study was reviewed by Mercy College's Institutional Review Board (IRB) and deemed qualified for IRB exemption. Student data was collected across three semesters from three sections of a 200-level Cell Biology course taught by the same instructor. Cell Biology is a required course for biology majors and is typically taken during the sophomore year. Each section had between 14 – 18 students and the total number of students enrolled across the three semesters was 47 (83% were biology majors; 13% health sciences majors; 4% other). Prior to enrolling in Cell Biology, students are required to complete a year of General Biology and typically a year of General Chemistry. Students' responses to the course were anonymous and collected at the end of each semester from the Student Assessment of Learning Gains (SALG) survey tool (https://salgsite.net/). The response rate was 79%.

Students engaged in an authentic research experience to investigate changes in the actin cytoskeleton in primary astrocyte cells. Individual students performed the optic tectum dissection and worked in groups of three to dissociate the tissue and plate the primary cells (see below). Each group chose a drug of their interest, for example: caffeine, melatonin, sertraline, diazepam, zolpidem, yoda1, ethanol, carbaryl, tetracaine, and .capsaicin (all purchased from Sigma Aldrich) and graviola extract (purchased from GNC health store). The students analyzed the scientific literature, developed a hypothesis based on the literature and designed a novel study to investigate the effects of their drug of interest on astrocytic actin. Students carried out their experiments on confluent primary astrocyte cultures (generated approximately two weeks prior). Following drug treatment, astrocytic actin structure was analyzed using a phalloidin-rhodamine staining technique (see below). Students performed, on average, two replicates of their experiments.

Learning Objectives

The learning objectives of the authentic astrocyte-cytoskeleton research project are to:

1. Work collaboratively to develop a research project investigating actin changes in glial cells in response to a drug of interest
2. Develop a hypothesis (based on a primary research article) regarding actin reorganization in astrocytes
3. Design an experiment to test the hypothesis
4. Gain proficiency in chick embryonic brain dissection, enzymatic dissociation of primary glial cells, aseptic technique, fluorescence staining techniques, and phase contrast and fluorescence microscopy
5. Calculate molarities, create working solutions, and carry out drug treatments
6. Interpret experimental results involving phalloidin fluorescence staining
7. Describe the cellular mechanism(s) that facilitate changes in cytoskeletal structure
8. Create an abbreviated scientific manuscript containing abstract, introduction, materials and methods, results, and conclusion sections
9. Present the background literature and results of the cytoskeleton research project

Materials Required for Primary Astrocyte Culture Preparation

- Fertilized chicken eggs
- Egg incubator
- Dissecting microscope
- Sterile small dissection scissors
- Sterile fine tip forceps
- Sterile curved tip forceps
- Sterile gauze
- Laminar flow hood
- Trypsin LE
- Dulbecco's Modified Eagle's Medium (D-MEM)
- Chicken Serum
- Fetal Bovine Serum
- Penicillin Streptomycin
- DNase
- 70 µm cell strainer
- CO_2 incubator
- Inverted microscope
- Phalloidin-rhodamine (optional, for actin staining)

Primary Astrocyte Culture Preparation

Preparation of Chick Embryos for Dissection

- Astrocyte cultures are prepared from embryonic day 8 chicken embryos.
- Fertilized eggs are obtained from a commercial source, such as Charles River Laboratories, MA.
- Store fertilized eggs at 12 C for 0 days – ~1.5 weeks prior to incubation. An inexpensive wine fridge (e.g., Ivation Wine Cellar) is sufficient for storage.
- To start development, place fertilized eggs in a humidified 38 C incubator (e.g., 1502 Sportsman Incubator) for 8 days.
- A sterile dissection hood is not necessary, but surfaces should be wiped down with 70% ethanol prior to dissection, gloves sprayed with 70% ethanol and clean lab coats worn.
- Candle eggs with a light source to verify viability by presence of vasculature (Figure 1A). Students can use their cell phone flashlights, positioned underneath egg.
- Spray eggs with 70% ethanol and crack egg open (on the side opposite the vasculature) into a sterile 10 cm petri dish.
- Empty contents of the egg into the petri dish, cut away

amniotic sac and vasculature with sterile small dissection scissors.
- Place embryo into clean petri dish and quickly decapitate with scissors.
- Use curved forceps to gently scoop up head and place onto sterile gauze (oriented with the dorsal side up and the anterior end facing away from experimenter - see Figure 1B).

Steps of Optic Tectum Dissection
- All dissection procedures should be performed using a dissection microscope (e.g., Olympus CKX41 dissecting microscope).
- Dissection images can be captured with a simple image capture system (e.g., Accu-Scope camera with image capture).
- With embryo head placed on sterile gauze, use fine tip forceps to open the developing layer of skin (cartilage and bone are not yet formed), fully exposing the optic tectum below (Figure 1B, C).
- Using curved forceps flank both sides of the optic tectum and pinch below the surface of the optic tectum directly above the midbrain (Figure 1D).
- Gently pull the optic tectum up and away from the midbrain, separating it completely from the midbrain, and place the optic tectum onto sterile gauze (Figure 1E). During the removal of the optic tectum, the meningeal layer may sometimes come off, however if not, it will need to be removed in a separate step.
- Figure 1E shows the optic tectum with meningeal layer. still present. Use fine tip forceps to push or slide the meninges until part of the meningeal layer adheres to the gauze (not shown).
- Gently push or roll the optic tectum away from the meninges, this will cause the remainder of the meninges to stick to the gauze, effectively removing the entire meningeal layer from the optic tectum (Figure 1F).
- Care should be taken to remove the entire meningeal layer, as meningeal cells will grow rapidly and greatly reduce the purity of the astrocyte culture.
- Once the meninges is removed, place the clean optic tectum in cold Ca^{2+}/Mg^{2+} free 1x phosphate-buffered solution (PBS).

Steps Of Tissue Dissociation
- Ideally, the tissue dissociation will take place under a laminar flow hood. For our Cell Biology class, we use tabletop portable Sentry Air Systems (Cypress, TX) laminar flow hoods (18" or 24").
- Remove the optic tectum from the cold PBS and place in 10 mL of prewarmed (37 C) 1x trypsin LE (Gibco) solution, prepared in Ca^{2+}/Mg^{2+}-free Earle's Balanced Salt Solution, and incubate in 37 C water bath for 10 min.
- After the 10 minute incubation, add 15 µL DNase (Sigma) to break down DNA released from damaged cells (which would otherwise clump the tissue together).
- Centrifuge the tissue for 5 min at 1,100 rpm.
- Decant the supernatant and resuspend the pellet in 10 mL of plating media (D-MEM with 5% chicken serum, 5% fetal bovine serum, and 0.5% penicillin/streptomycin). All cell culture reagents are from Gibco. Trypsin inhibitor is not necessary, as the serum will inhibit trypsin activity.
- Centrifuge the cell suspension again at 1,100 rpm for 10 min; again resuspend the pellet in 10 mL plating media.
- Pass the cell suspension through a 70µm cell strainer.
- Count cells and plate at a density of approximately 250,000 cells per mL onto Primaria® plates or dishes (Corning) or Permanox chamber slides (Thomas Scientific).
- Maintain cells in a humidified 5% CO_2 incubator.
- Replace cell media the day after initial plating and twice a week thereafter.
- Cells should reach confluency approximately 1.5 – 2 weeks after plating and are typically viable for up to one month.
- If microglial cells overgrow the culture (small phase bright cells that float or loosely on top of astrocytes), confluent astrocytes can be treated with the mitotic inhibitor 8 µM Ara-C (Sigma) for 3 days to reduce microglial growth.
- Cells can be imaged with an inverted microscope (e.g., Olympus CKX41) with simple image capture system, such as Accu-Scope camera with image capture screen.

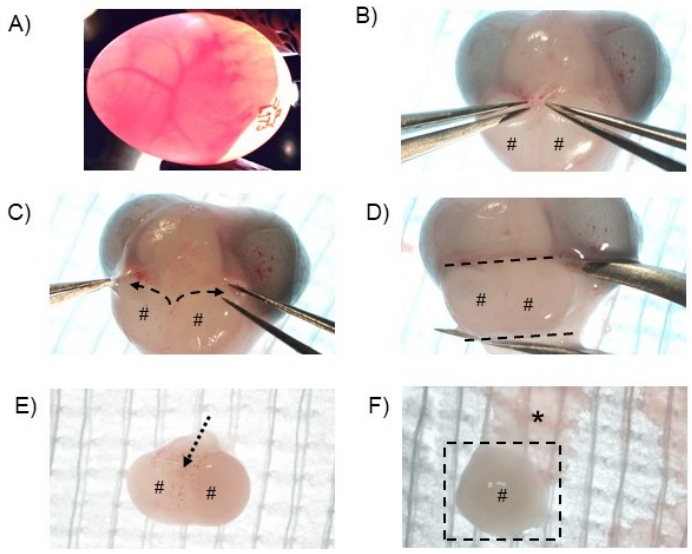

Figure 1. Dissection of optic tectum (OT). # indicates location of OT. *A)* Candled egg showing vasculature of embryonic day 8 chicken embryo. *B)* To remove the developing skin, forceps are placed between the two hemispheres of the OT. The skin is pinched with forceps and pulled laterally, as shown in *C)*, to expose the OT (arrows indicate pulling of skin in lateral direction). *D)* Curved forceps are placed on either side of OT (dashed line represents boundary of OT and position of curved forceps). Curve forceps are depressed towards bottom of OT and OT is pinched on bottom surface and removed, leaving midbrain in place (not shown). *E)* OT is placed on clean gauze, meninges covering is shown (arrow). Meninges is removed by using forceps to slide or push it towards gauze and pushing or rolling OT away from meninges (not shown). *F)* Meninges is shown stuck to gauze (arrow) and clean OT hemisphere remains (box).

Steps of Actin Staining

- Phalloidin-rhodamine (Invitrogen), a non immunostaining technique, can be used to stain for F actin.
- Astrocytes plated on chamber slides are recommended for this procedure.
- Wash cells 1x with PBS.
- Treat cells with 4% paraformaldehyde for 10 min.
- Wash 3x with PBS for 5 minutes each on slow speed shaker.
- Permeablize cells by replacing PBS with 0.25% triton-x-100 for 30 minutes on slow speed shaker.
- Remove triton x-100 and treat cells with 0.2 μM phalloidin-rhodamine (prepared in PBS).
- Wash cells 2x with PBS followed by slide mounting with ProLong™ Gold Antifade Mountant with DAPI (Invitrogen).
- Capture fluorescence images with, for example, Olympus BX51 upright fluorescence microscope and Infinity 3 camera.

RESULTS

To prepare the primary astrocyte cultures, each student individually performed a chick optic tectum dissection and performed the tissue dissociation in groups of three students. Students carried out both the dissection and dissociation within a 3-hour lab period. After the instructor demonstrated the dissection technique on an overhead video monitor, students were assisted with the dissections by both the instructor and a senior student teaching assistant. Novice students (those with limited laboratory experience and no dissection experience) typically take

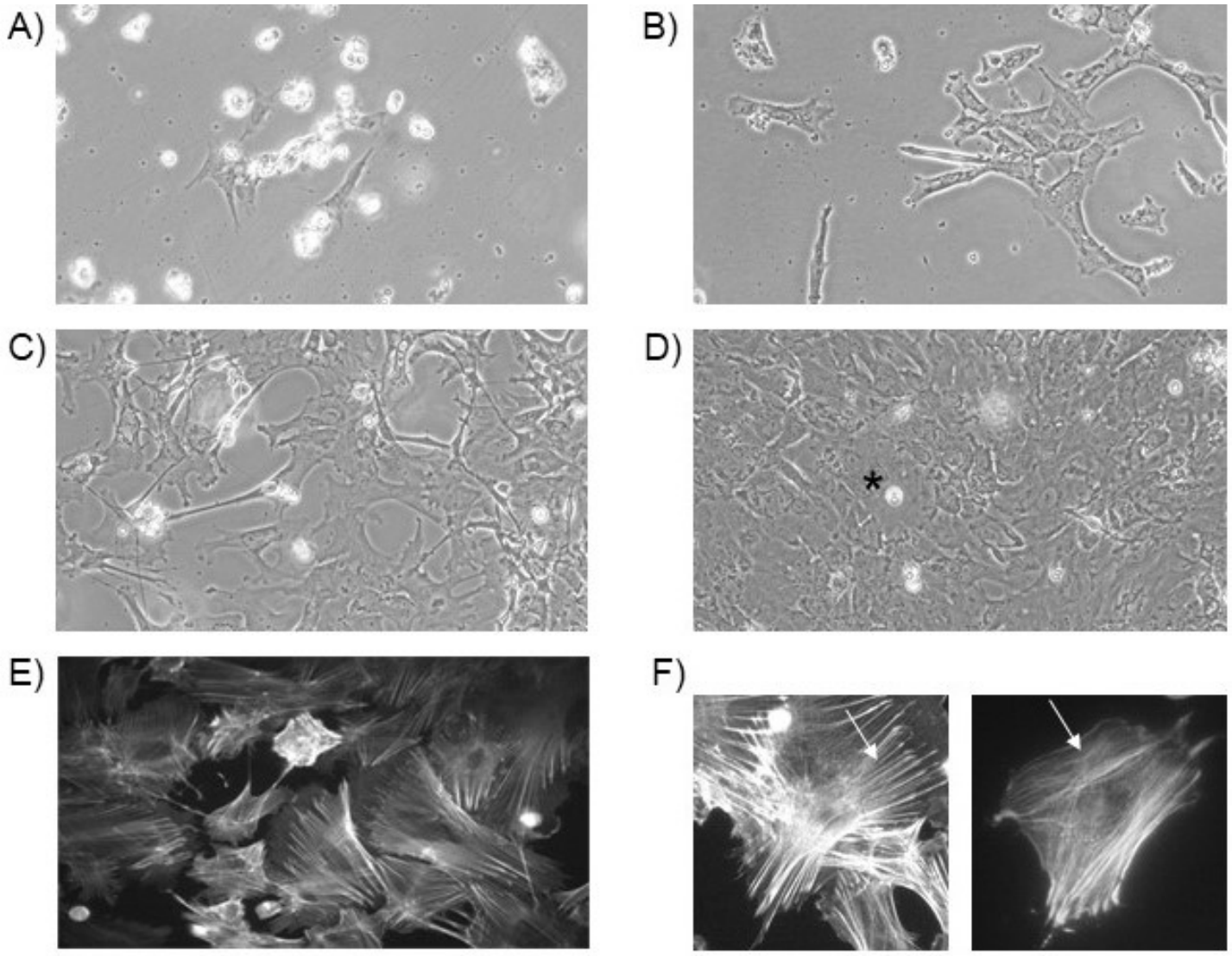

Figure 2. Primary astrocytes derived from embryonic day 8 chicken embryo optic tectum. *A)* Primary glial cells 1 day *in vitro* (DIV). Flat cells in center of well are precursor astrocytes. Bright white cells are debris or microglia. *B)* Primary astrocytes 6 DIV. *C)* Primary astrocytes 8 DIV, example of 80% confluent monolayer. *D)* Primary astrocytes 8 DIV, example of confluent monolayer with sparse microglia (as indicated by *). *E)* Phalloidin-rhodamine actin staining of untreated primary astrocytes. *F)* Phalloidin-rhodamine actin staining of untreated astrocyte culture (left panel) and astrocyte culture treated for 1 hr with 20 μM zolpidem. Arrows indicate astrocyte F-actin fibers.

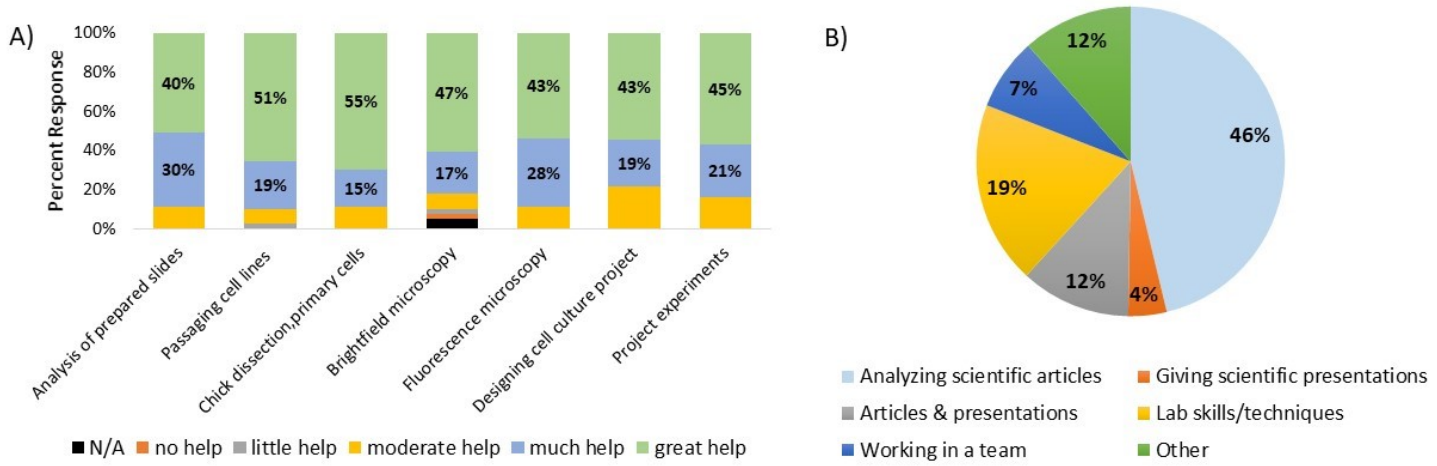

Figure 3. Student perceptions of their learning gains. Students completed the Student Assessment of their Learning Gains (SALG) survey N = 37. *A)* Students responded to the question: *HOW MUCH did each of the following aspects of the class HELP YOUR LEARNING?* using a Likert scale with a range from "no help" to "great help". The percent response for each leaning activity is shown. *B)* Students responded to the free response question: *Please comment on what SKILLS you have gained as a result of this class?* Answers were coded for the represented categories and reported as percent response.

between 40 minutes – 1 hour to complete a single optic tectum dissection. Despite the slow dissection time, all students in the Cell Biology course succeeded in preparing viable cultures. Figure 2 shows representative astrocyte cultures prepared by an undergraduate student.

At 1 day *in vitro* (DIV) (Figure 2A), a low density of precursor astrocytes can be seen in the center of the plate along with several dead cells/debris or microglia floating in the culture. A low plating density is ideal in that it cuts down on microglial contamination, supporting an enriched astrocytic culture. A lower density also reduces the number of neurons, which do not adhere to non-coated culture plates/dishes but can adhere to astrocytes (plating astrocytes at a low density limits the adherence sites for neurons). By 6 DIV (Figure 2B), astrocytes show signs of division and by 8 DIV astrocytes are between 80% to 100% confluent (Figure 2C,D).

The astrocytes grow in culture for two weeks prior to the students carrying out their experimental studies. During this two week period, students monitored the growth of their astrocytes through microscopic image capture and finalized the design of their experiments. In groups of three, students analyzed the scientific literature and designed a novel study exploring the effect of a drug of their interest on the actin cytoskeletal architecture. Since the primary astrocyte cultures are viable anywhere from 4 to 5 weeks post culture prep, experimental replicates of two are easily obtainable from one astrocyte prep.

Students most often investigated compounds familiar to them. For example, compounds found in drinks or foods (e.g., ethanol, caffeine, melatonin, capsaicin, graviola extract); anti-depressants, hypnotics or anesthetics: (e.g., sertraline, zolpidem, tetracaine); and pesticides (e.g., carbaryl). After determining appropriate concentrations from the scientific literature, students performed calculations, made working solutions of their drugs and treated the astrocyte cultures in serum containing medium for short durations (e.g., 1 hr) or longer durations (e.g., 24 - 48 hours) before fixing cells in paraformaldehyde for later phalloidin-rhodamine staining.

Figure 2E shows a representative phaoidin-rhoadmine staining of untreated astrocytes performed by an undergraduate student. Students were instructed to look for qualitative changes in the F-actin organization. Figure 2F shows an example experiment demonstrating F-actin reorganization in response to zolpidem treatment. Parallel actin filaments are observed in the control culture (Figure 2F, left panel, 20 µM zolpidem) and 1 hour 20 µM zolpidem treatment (Figure 2F, right panel) is shown to decrease the parallel fiber organization. Of note, while all students obtained successful phalloidin-rhodamine images, several lab groups either observed no changes in actin cytoskeletal organization in response to their drug treatments or observed toxicity, providing opportunities for students to critically evaluate their negative results and plan future experimental directions (as discussed in their final abbreviated scientific manuscripts).

The pedagogical model described here has elements of both a CURE and PBL. It follows a CURE model in that novel experiments are designed with the intent of generating new data of interest to the scientific community. It also follows elements of a PBL model in that students drive the direction of the project and design their own experiments even though they are at a novice stage (i.e., they choose the experimental drug and concentration/duration of the drug treatments). While students did not present their research outside of class, they each produced a short scientific manuscript and an oral presentation which covered both their research results and analysis of the journal article on which they based their studies. The average grades across the three sections of Cell Biology were: scientific manuscript, 87% +/- 6.8 SD; presentation of article, 87% +/- 6.3 SD; and overall grade for the course, 83% +/- 8.5 SD.

Student SALG responses showed positive learning gains from the authentic neuroscience research experience. In response to the question, *HOW MUCH did each of the*

Please comment on what SKILLS you have gained as a result of this class.
I am able to interpret and analyze scientific articles in a more effective way.
I learned how to read scientific articles more effectively.
This was the first class ive had to read a scientific paper in so doing that a couple times definitely had me step outside my comfort zone and gain a little skill.
I have gained skills in how to approach reading and interpreting scientific journals, as well as how to effectively translate the information to others.
I have a better understanding on how to properly break down and process scholarly journals and articles.
I was able to learn how to conduct my own experiment.
I gained a ton of lab experience and skills using the microscopy and how to run a sterile lab
Working as a team and helping each other understand anything we might have not understood because sometimes when someone else explains you might understand better.
I learned a little on how to read and interpret an article, but I still struggled with it.

Table 1. Representative SALG survey responses to the question: "Please comment on what SKILLS you have gained as a result of this class."

following aspects of the class HELP YOUR LEARNING?, more than half of the students indicated that dissecting embryo brains and preparing astrocytes was of "great help" to their learning, more so than any of the other activities surveyed (Figure 3A). Of note, prior to the primary dissections, students performed two laboratories in which they learned to passage cell lines, which also seemed to have had a positive impact on their learning.

When responding to the free response question, *Please comment on what SKILLS you have gained as a result of this class,* nearly half of the class indicated a positive learning gain from analyzing the scientific literature, a major component of their student-driven research project. Twenty-percent responded that lab technique/experimental design skills supported their learning and 12% reported that both analyzing scientific articles and presenting their findings added to their skill set. Table 1 shows example responses to, *Please comment on what SKILLS you have gained as a result of this class,* and Table 2 shows free responses to, *Please comment on how the CLASS ACTIVITIES helped your learning.* Of these responses regarding how class activities helped students' learning, 36% of respondents indicated lab related items as activities that greatly impacted their learning (not shown).

DISCUSSION

We describe here the detailed steps for producing high quality astrocyte brain cultures from a chicken embryo, a cost effective model that does not require an animal facility and can be easily accommodated at a primarily teaching institution. This technique can be accomplished by undergraduates in a 3 hour laboratory class, with viable cells being produced even from those performing the technique for the first time. We show that these cultures are ideal for authentic research investigating actin changes using the phalloidin-rhodamine technique. Additional techniques exploring the cytoskeleton are also possible. For example, the Cell Biology students perform immunocytochemical staining of beta-tubulin earlier in the semester. However, because immunocytochemical beta-tubulin staining is more time intensive and the staining results more variable compared to phalloidin-rhodamine, phalloidin staining was better suited for the student-research projects.

Primary astrocyte cultures are ideal for a variety of laboratory exercises in addition to exploring cytoskeletal changes. Astrocytes are rich in the antioxidant glutathione and thus facilitate robust neuroprotection (Haskew-Layton et al., 2010; Haskew-Layton et al., 2013). Investigating glutathione changes in response to drug treatments using the fluorescent dye monochlorobimane or exploring astrocytic-neuroprotection using a neuron-specific MAP2 ELISA method (potentially for more advanced students) would provide an impactful framework for a CURE. Additionally, monitoring cytokine and chemokine responses in response to the pro-inflammatory molecule lipopolysaccharide (LPS) is another potential use for astrocyte cultures in an undergraduate teaching laboratory (Xie et al., 2016).

We feel that the preparation and use of primary astrocyte cultures for authentic research is ideal for novice students with minimal laboratory experience. While a previous study demonstrated successful preparation of chick embryonic primary neurons by undergraduates in a research course (Burdo, 2013), these students had prior course and/or laboratory experiences in Introductory Neuroscience, Cell Biology and Investigations in Molecular Biology. In contrast, our method of astrocyte preparation can be readily mastered by novice sophomore students with limited prerequisite coursework (e.g., General Biology and General Chemistry) and minimal laboratory experience (e.g., most of our students had no experience with dissections or micropipetting prior to enrolling in Cell Biology). In fact, our

Please comment on how the CLASS ACTIVITIES helped your learning.
I enjoyed the lab portion of this class, I learned a lot
These hands on activity were every [sic] valuable throughout the class because just watching others do these procedure doesn't help you remember by actively participating will help you get a better understanding of the class.
Cell Passaging was of great interest. Did not know anything about cell passaging until it was taught in class. Great learning and hands-on experience.
All of the hands on lab work was extremely beneficial as doing is the only true way to learn experimental practices. One thing I wish we did was go over the images we did capture at a more in depth level.
Loved that we were able to use chick embryos for out experiments
Labs were a great way to develop skill
Applying what we learned in class to lab helped a lot in understanding material
I loved being able to carry out my own individual experiment. This really helped me to apply what I had been learning all semester.

Table 2. Representative SALG survey responses to the question: "Please comment on how the CLASS ACTIVITIES helped your learning."

students prepare successful primary astrocyte cultures on their first attempt with no prior dissection practice. The key to successful astrocyte preparation by novice students is likely due to the increased tolerance of glial cells over neuronal cells to long dissection times and the use of a sterile gauze technique to easily remove meningeal cells (removal of the meninges with the traditional forceps method is technically challenging).

While Burdo (2013) uses day 8 chicken embryos to culture neurons, this developmental stage is rich in radial glial cells (Seo et al., 2008) which differentiate into astrocytes under culture conditions (Culican et al., 1990). We find that the embryonic day 5 – 6 chick optic tectum is ideal for preparing enriched neuronal cultures (seeded on poly-d-lysine coated plates) that contain minimal glial contamination (unpublished observations). However, this earlier development stage requires more precise dissection skills and is better suited for students with some dissection experience (unpublished observations). We use day 8 chick embryos for astrocyte cultures because the optic tectum is easy to dissect at this stage and later developmental stages are sometimes distressing for novice students to work with. Our use of uncoated tissue culture plates (e.g., no poly-d-lysine) and a low initial plating density favor an astrocyte-enriched culture as observed approximately 1 - 2 weeks post plating.

In summary, students reported positive learning gains from the laboratory experiences. Cell culturing exercises had a strong impact on student learning while more traditional labs involving analysis of prepared slides had the smallest impact on learning (Figure 3A). In addition to the laboratory exercises, students reported strong learning gains from engaging in aspects of the scientific method related to analyzing scientific articles and building upon a body of existing scientific evidence to design and conduct an authentic research study (Figure 3B, Tables 1 and 2). In conclusion, primary chick embryonic astrocytes can be successfully prepared by undergraduates with minimal laboratory experience, providing an impactful experimental model for undergraduates to develop authentic neuroscience research projects.

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