2:186 Neurobiology Lab

Week 6 (Feb. 23): Neuroprogenitor cell culture techniques.

Lecture: Culturing neuronal and glial cells; sterile technique demonstration.

Lab Learning Objectives: After completing the exercise you should be able to…

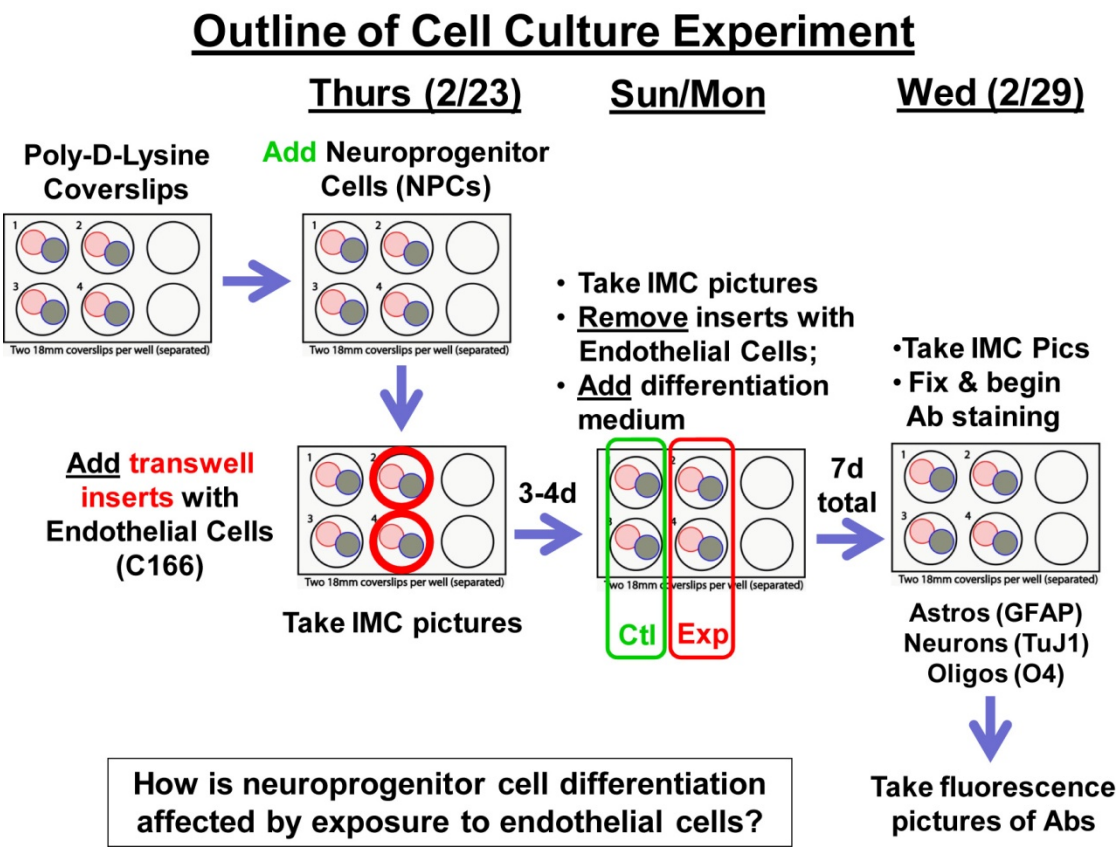
- Identify the advantages and disadvantages of *in vitro* cell and tissue culture.
- Use sterile technique to prepare substrata (glass coverslips), culture media, and triturated cells, place multipotent progenitor cells in culture, and carry them for several days without contamination.
- Use an inverted microscope to capture transmitted light images of live cells in culture to monitor their health and development.
- Use indirect immunofluorescence antibody staining to identify different types of cells (neurons and glia) in dissociated cell cultures.
- Determine the effect of co-culturing endothelial cells on neuroprogenitor cell differentiation.

Grading:

Lab report on cell cultures. DUE date and guidelines for the report will be distributed later.

Handouts:

1. "Preparation of coverslips."
2. "Starting Co-Cultures of Endothelial and neuroprogenitor cells."
3. "Recipes for media."



Lab Exercises:

Thursday (Feb. 16):

__ 1. Prepare culture substratum by adding adhesive solution (e.g., poly-lysine) to coverslips. See **Handout #1**.

Thursday (Feb. 23):

__ 2. Complete the final steps in preparing the substratum (coverslips). See **Handout #2 (Step 1).**

__ 3. Demonstration on adding neuroprogenitor cells (NPCs) to coverslips to start cultures. See **Handout #2.**

__ 4. Prepare and plate NPCs onto coverslips in 4 wells. See **Handout #2.**

__ 5. Switch endothelial cells (C166) on transwell inserts to **neuroprogenitor medium**.

__ 6. Place 2 transwell inserts (carrying endothelial cells) on top of 2 neuroprogenitor cell culture wells.

__ 7. **Demonstrations** on (1) phase contrast microscopy to check cells and (2) capturing IMC (integrated modulation contrast) images of cells using a Leica DM-IL microscope (located in the tissue culture suite).

__ 8. **Take pictures**. Use the Leica DM-IL microscope (in tissue culture suite) to take an IMC (transmitted light) image of cells in all 4 wells at starting point of culture. Save the images for your report.

__ 9. Prepare **neuronal differentiation medium** for use on Sun/Mon. See **Handout #3**.

Sunday (Feb. 26) or Monday (Feb. 27):

__ 10. Remove the 2 transwell inserts.

__ 11. Switch all wells from neuroprogenitor medium to **neural differentiation medium.**

__ 12. Use the Leica DM-IL microscope to capture images of your cultured cells (all wells). Be careful not to disturb or contaminate the cultures.

Wednesday (Feb. 29):

__ 13. Take representative IMC images of cells in all wells (before fixing).

__ 14. Fix coverslips in all wells.

Thursday (Mar. 1):

__ 15. To determine the phenotype and density of cells, perform immunocytochemistry with the following antibodies:

a. anti-TuJ1 (neurons)
b. anti-GFAP (astrocytes)
c. anti-O4 (oligodendrocytes)

Counter-stain with DAPI to label nuclei of all cells. Capture images using fluorescence scopes.

Materials/Equipment:

- Protective gloves of various sizes (and masks, gowns/lab coats, if desired)
- Cells for culture (neurospheres obtained from frozen stocks or brainbits.com)
- Cell culture CO2 incubator
- Culture medium
- 10ml Borate buffer
- Aliquots of poly-D-lysine
- 10ml syringes
- Syringe-tip filter units (yellow, 0.2 um)
- Autoclaved forceps in silver autoclave dishes
- Autoclaved coverslips (18mm round or 22mm square)
- Sterile pipetman tips
- Tubes (15 ml)
- 70% ethanol squash bottle

Reference Materials:

Banker, G., and K. Goslin. "Types of Nerve Cell Cultures, Their Advantages and Limitations." In, Culturing Nerve Cells, Second Edition. MIT Press, 1998.

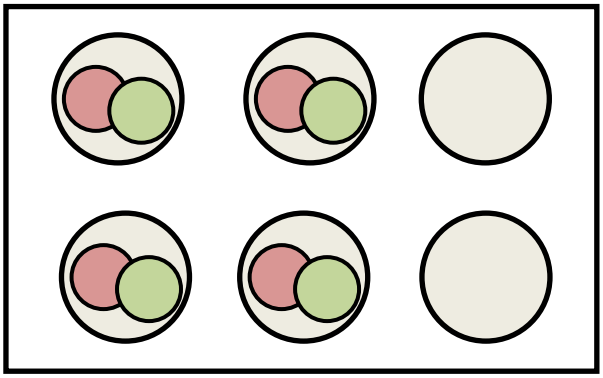
Barker, K. "Chapter 9: Working without Contamination." In, At The Bench: A Laboratory Navigator. Cold Spring Harbor Laboratory Press: New York. Pp. 185-203.

Preparation of coverslips

Step 1: Place coverslips in a 6-well plate.

Place two 18-mm round glass coverslips in each of 4 wells in a 6-well plate (total of 8 coverslips) using sterile forceps.

Replace the cover, and start making the PDL adhesion solution.



Step 2: Make 10 ml of poly-D-lysine solution (used to coat the coverslips).

You will be provided with a stock solution of Poly-D-Lysine (PDL) at a concentration of 1 mg/ml.

Dilute the stock PDL 1:10 in provided Borate buffer to a final concentration of 100 μg/ml. Make a total of 10 ml of diluted PDL in Borate buffer.

Filter-sterilize the solution using a sterile Millex yellow filter unit (pore size = 0.2 μm) attached to a 10-ml syringe.

Step 3: Apply the PDL solution to the coverslips.

Add 2.0 ml of diluted PDL to each of the four wells containing coverslips, making sure the coverslips are not on top of each other, and seal the edge of the plate with parafilm to prevent drying out.

Incubate coverlips in PDL solution at $4^{o}C$ (refrigerator) until next Thursday.

On Thursday, rinse wells two times with sterile H_2O (2 ml/rinse) and keep with 2 ml of medium 'A' until ready for use.

Handout #2: Starting co-cultures of endothelial and neuroprogenitor cells

Step 1 - Performed under t/c enclosures:

Prepare tissue culture enclosures:

- **Clean** with 70% EtOH, treat with UV (10-15 min).
- **Warm** up medium A in water bath (37°C).
- **Discard** the PDL solution from the plate.
- **Rinse** wells with sterile water (~2 ml/rinse) two times, and **keep** with fresh 2 ml of **Progenitor** medium A for 1-4 h. Be careful not to displace coverslips.

Step 2 - Performed under t/c enclosures:

- **Obtain** a plate with Progenitor cells from Olga: ~240,000 cells/well in **Progenitor medium A**.
- **Collect** neuroprogenitors from **3 wells** in a sterile tube (total volume ~6 ml).
- Carefully **triturate** your cells: [Be careful not to generate air bubbles, as this will damage cells.] 10 times using two borosilicate fire-polished Pasteur pipet (several different pasteur pipets will provide better cell dissociation).
- **Add** 5 ml of fresh **Progenitor medium A** to cells, gently **mix** by pipetting 2-3 times.

Step 3 - Performed under t/c enclosures:

- **Discard** medium from four wells with coverslips.
- **Add** progenitors to wells #1-4, 2.5 ml per well. **Cover** the plate and carefully evenly **distribute** the cells without displacing the coverslips.
- **Place** the plate in the tissue culture incubator (37^{o}C, 5% CO_2).

Step 4 - Performed under t/c laminar flow hood (in t/c suite):

- Obtain a plate with endothelial cells (C166) plated in transwell inserts from Olga ($5x10^5$ C166 cells per insert).
- To change medium in C166 cells to progenitor medium, add 2.5 ml of **Progenitor medium A** to two new wells and transfer two inserts to these wells using sterile forceps.
- Discard endothelial cells medium from inserts using 5-ml pipette and add 1.5 ml of **Progenitor medium A.**
- **Place** the insert with C166 cells into the plate with progenitor cells (2 experimental wells).
- **Place** the plate in the tissue culture incubator for 3-4 days.

Step 5 - Performed under t/c laminar flow hood on Sunday:

- Warm up **Neuronal Differentiation Medium B.**
- Remove the 2 transwell inserts and discard.
- Carefully discard Progenitor medium from wells with coverslips using 5-ml pipette and add warm **Neuronal Differentiation Medium B**, 2 ml per well .
- **Place** the plate in the tissue culture incubator for 3-4 days.

Handout #3: Recipes for Media

Progenitor medium (A): PROVIDED

- NEUROBASAL™ Medium (NB)
- 2% B-27 Supplement Minus Vitamin A
- 20 ng/ml hFGF2
- 20 ng/ml hEGF
- 0.5 mM Glutamax
- 10 μg/ml Gentamicin

Filter-sterilized, 0.2 μm filter pore size

Neuronal Differentiation medium (B): total volume of 10 ml	VOLUME
• NEUROBASAL™ Medium (NB)	9.725 ml
• 2% B-27 Supplement Minus Vitamin A (B27)	0.2 ml (50X stock)
• 0.5 mM Glutamax	25 μl (200 mM stock)
• 50 U Penicillin and 50 μg Streptomycin /ml	50 μl (10,000U/ml Pen and 10,000μg/ml Strep stock)

Filter-sterilize, 0.2 μm filter pore size

Keep in the refrigerator until use.

Medium for C166, yolk-sac derived endothelial cells from a hypervascular transgenic mouse:

DMEM (GIBCO, with Glucose, 4.5 g/L, + L-Glutamate, - Sodium pyruvate), 5% FBS (fetal bovine serum), 1% Pen/Strep.

GFAP and TuJ1 Immunocytochemical (ICC) Staining of Cell Cultures on Coverslips

Summary of Procedure					
✓	**Step**	**Procedure**	**Time**	**Total Time**	**Stop point**
Do not let coverslips dry out at any point of staining. Steps 1-5 are done in the 6-well plate. For each step, remove the previous solution before adding next.					
	1	Place coverslips in 6 well plate, 1 coverslip/well. Briefly wash in PBS (to remove any serum proteins)	~10 sec	0:00	
	2	Fix in formaldehyde (4% in PBS). Dispose fix in separate waste.	10 min	0:10	
	3	PBS wash (2 washes @ 2.5 min each). Dispose with fix waste.	5 min	0:15	STOP
	4	Extract and block (1% Triton X-100 & 20% serum in 1XPBS)	15 min	0:30	
	5	Wash (0.1% Triton X-100 & 1% serum in 1XPBS)	5 min	0:35	STOP
For Step 6, remove *only **one** coverslip from each condition* and place (cells side up!) on a piece of Parafilm inside a 150mm plastic petri dish (humidified chambers). Wet the filter paper inside the petri dish. Label the Parafilm with a marking pen to keep track of coverslips. Apply the Ab solutions to the top of the coverslips (~3 drops) and cover.					
	6	**Experimental:** Primary (1^{o}) Antibody incubation – at room temperature. **Control:** Add antibody diluent to the control coverslip (in the well). When this step is done, carefully pick up the coverslips and blot edge on a paper towel to remove the solution.	45 min	1:20	
Step 7 is done in the 6-well plate. Add 2.0 ml of 0.1% Triton X-100 & 1% serum buffer to wells and transfer coverslips to these wells. Label wells properly.					
	7	Wash in 0.1%Triton X-100 & 1%serum buffer (3 washes @ 5min ea)	15 min	1:35	STOP
Step 8 is done on Parafilm as in Step 6. Blot on paper towel when complete.					
	8	Secondary (2^{o}) Antibody incubation (in the dark)	45 min	2:20	
Step 9 is done in the 6-well plate. Add 2.0 ml of fresh 0.1% Triton X-100 & 1% serum buffer to wells and transfer coverslips to these wells.					
	9	Wash in 0.1%Triton X-100 &1% serum buffer (2 washes @ 5 min each) – in the dark	10 min	2:35	STOP
	10	Add diluted DAPI solution (1:300 in 1XPBS) to wells for 10-20 min in the dark	10 min	2:45	
	11	Wash all coverslips in the wells with 1XPBS (2 washes @ 10-15 min each) in the dark shaking	30 min	3:15	STOP
	12	Optional: Rinse coverslips with ddH_2O, air dry	3 min	3:18	
	13	Mount coverslips on glass slides using Cytoseal. Label. Store at $4^{o}C$. It is best to take pictures within 24hr, but staining should last for weeks if kept in cold and dark.			

STOP **"Stop sign" means that after this step, specimens can be covered with 1XPBS and stored covered with solution (or in a humidified chamber, as needed) at $4^{o}C$ until next step.**

Materials needed:

Phosphate buffered saline (PBS)
Fixative (16%, stock solution)
Triton X-100 (10 % stock solution)
Serum
Primary Antibodies (GFAP; TuJ1)
Fluorescent Secondary Antibodies (Alexa Fluor-488 goat anti-rabbit IgG and Alexa Fluor-568 goat anti-mouse IgG)
Glass slides
Mounting medium, Cytoseal

Solutions needed:

PBS (>25 ml)

Fixative (4% formaldehyde in PBS) (16 ml)

8 ml of 2XPBS, 4 ml of 16 % formaldehyde, 4 ml of H_2O. Vortex the solution to mix thoroughly.

Fixative (16% formaldehyde in PBS) (40 ml) - make fresh

17.3 ml 37% formaldehyde, 20 ml 2XPBS, 2.7 ml H_2O, mix thoroughly.

1%Triton X-100 & 20% serum in 1XPBS (10 ml)

5.0 ml of 2XPBS, 2 ml of serum, 1.0 ml of 10% Triton X-100, 2 ml of H_2O. Vortex solution.

Buffer (40 ml) - contains 0.1% Triton X-100 and 1% serum in 1XPBS

39.2 ml of 1XPBS, 0.4 ml of serum, 0.4 ml of 10% Triton X-100. Vortex solution.

Primary and Secondary Antibody Solutions

1^o Abs: Dilute primary Abs, anti-TuJ1 **and** rabbit anti-GFAP, 1:50 in Buffer (**4 µl of each antibody** per **200 µl** of **0.1% Triton X-100 and 1% serum** buffer), and vortex to mix.

2^o Abs: Dilute Alexa Fluor-488 goat anti-rabbit IgG **1:50 in Buffer - 8 µl of antibody** per **400 µl** (apply at least 100 µl per coverslip), add Alexa Fluor-568 goat anti-mouse IgG (**1:25 dilution)** to the **same vial (16 µl).**

Dilute DAPI in 1XPBS (1:300).

Summary of O4 Immunostaining Procedure					
✓	**Step**	**Procedure**	**Time**	**Total Time (hr:min)**	**Stop-point**
Steps 1-5 are done in the 6-well plate. For each step, remove the previous solution before adding next.					
	1	Place coverslips in 6well plate, 1 coverslip/well. Brief wash in PBS (to remove any serum proteins).	~10 sec	0:00	
	2	Fix in formaldehyde (4% in PBS). Dispose fix in separate waste.	10 min	0:10	
	3	PBS wash (3 washes @ 5 min each). Dispose with fix waste.	15 min	0:25	STOP
	4	Blocking (5% serum in 1XPBS).	30 min	0:55	
	5	PBS wash (3 washes @ 5 min each).	15 min	1:10	STOP
For Step 6, remove *only **one** coverslip from each condition* and place the coverslip (cells side up!) on a piece of Parafilm inside a 150mm plastic petri dish. Label the Parafilm with a marking pen to keep track of coverslips.					
	6	Primary (1^{o}) Antibody incubation at $37^{o}C$ Apply the 1^{o} Ab solutions (100 µl/coverslip) to the top of each coverslip and cover the petri dish. When this step is done, carefully pick up the coverslips and blot the edge of the coverslip on a paper towel to remove the Ab solution. Add an antibody diluent to the control coverslip (in the well).	60 min	2:10	
Step 7 is done in the 6-well plate. Add 2.0 ml of fresh 1X PBS to wells and transfer coverslips previously in 1^{o} Ab to these wells. Label wells properly.					
	7	PBS wash (3 washes @ 5 min each). Be sure that coverslip is completely covered with the solution (2.0 ml per well).	15 min	2:25	STOP
Step 8 is done on Parafilm (as in Step 6). Transfer all coverslips to Parafilm and add 2^{o} Ab solution.					
	8	Secondary (2^{o}) Antibody incubation at $37^{o}C$, in the dark. When complete, blot edge of coverslip on paper towel to remove solution.	60 min	3:25	

Step 9 is done in the 6-well plate. Add fresh 2.0 ml of 1X PBS to wells and transfer coverslips to separate wells.					
	9	Wash all coverslips with PBS (1 wash @ 5 min) in the dark.	5 min	3:30	STOP
	10	Add diluted DAPI solution (1:300 in 1XPBS) to wells for 10-20 min in the dark	10 min	3:40	
	11	Wash all coverslips in the wells with 1XPBS (2 washes @ 15 min each) in the dark shaking	30 min	4:10	STOP
	12	Optional: Rinse coverslips with ddH_2O, air dry	3 min	4:13	
	13	Mount coverslips on glass slides using Cytoseal. Label. Store at 4^{o}C.			

STOP **Stop sign means that after this step samples (slides or coverslips) can be covered with 1XPBS and stored at 4^{o}C until next step. Make sure samples are completely covered with solution. Samples with small volumes of solution should be retained in a humidified chamber to prevent drying out.**

Solutions needed:

PBS (>25 ml)

Fixative (4% formaldehyde in PBS) (16 ml)

8 ml of 2XPBS, 4 ml of 16 % formaldehyde, 4 ml of H_2O. Vortex the solution to mix thoroughly.

Fixative (16% formaldehyde in PBS) (40 ml) - make fresh

17.3 ml 37% formaldehyde, 20 ml 2XPBS, 2.7 ml H_2O, mix thoroughly.

Blocking Solution (5% serum in 1XPBS) (20 ml)

10.0 ml of 2XPBS, 1.0 ml of serum, 9.0 ml of H_2O. Vortex solution.

Primary and Secondary Antibody Solutions (use at least 100 µl, should be sufficient for each 18mm coverslip)

Dilute Abs in **1XPBS**, and vortex to mix:

1) **Primary** Ab (1:100): mouse anti-O4 IgM (MAB345). Dilute **4 µl of antibody** per **400 µl** of 1XPBS.

2) **Secondary** Ab (1:50): Alexa Fluor-568 goat anti-mouse IgM**.** Dilute **8 µl of antibody** per **400 µl** of 1XPBS.

Dilute DAPI in 1XPBS (1:300).

How to Differentiate Between Different Types of Neural Cells in Culture

1) A good starting point is in knowing what stain you are looking at. Different stains label different types of cells:
   - Antibodies against **TuJ1**, a class III beta-tubulin, labels neurons and neural stem cells (fluoresces red in Fig. 1).
   - Antibodies to glial fibrillary acidic protein (**GFAP**) labels intermediate filaments in the cytoplasm of astrocytes (appears green in Fig. 1).
   - Antibodies against **O4** recognize an antigen on the surface of oligodendrocyte progenitors (red in Fig. 2 left).
   - Antibodies against **Iba1** (ionized calcium binding adaptor molecule-1), a calcium binding protein, labels macrophages and (in the brain) microglia.
   - Antibodies against **NG2**, a proteoglycan, labels polydendrocytes (also known as oligodendrocyte precursor cells, OPCs, or simply NG2 cells).

   These are only a few of the possible fluorescent stains you can use. Understanding your stain, the cells it targets and which part of the cell it stains is very important for distinguishing between cell types.

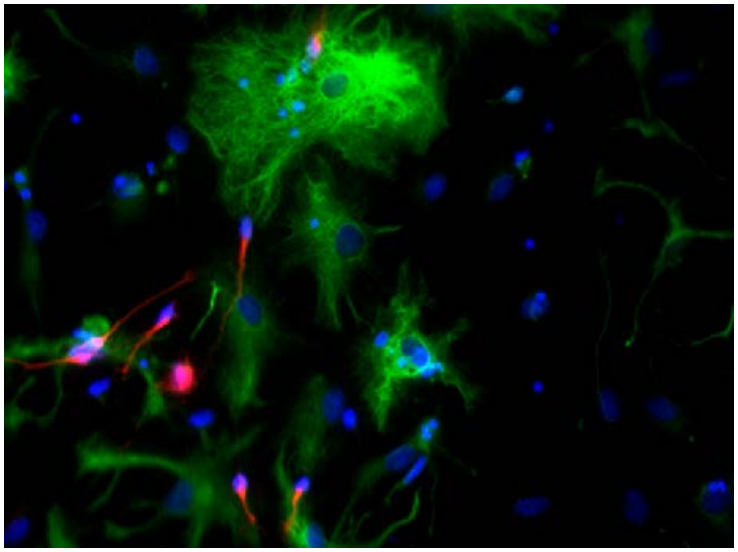
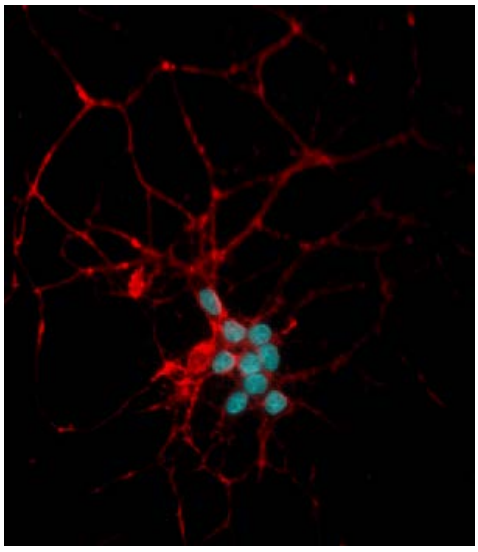


Fig. 1. This image of a neuroprogenitor cell culture preparation containing neurons and glia shows three different kinds of fluorescent stain. Astrocytes (green) are labeled with anti-GFAP antibodies. Neurons (red) are labeled with anti-TuJ1 antibody. DAPI (blue) labels the nucleus of all cells.

2) Another way to differentiate between types of cells is to understand their various morphologies. Astrocytes in culture (green in Fig. 1) are large, rather flattened cells. Their nuclei are large and faint compared to other types of cells (the large, blue DAPI-stained spots within the green of the cell). Neurons are smaller and have fewer projections, usually only one or two (red cells in Fig. 1). Their nuclei are smaller and brighter than astrocytes. Oligodendrocytes (pictured in Fig. 2, left) have many projections but appear less bushy than astrocytes. Their nuclei are medium in size and appear bright in DAPI stain. Microglia (right) look like very small astrocytes with much smaller nuclei. Combining their morphological features with the stain used is a great way to help determine cell types.
3) It is also important to be able to tell the difference between a living and a dead cell. DAPI staining is very helpful in this because the nuclei of dead cells are small, round, and bright, while the nuclei of living cells are larger, usually less brightly stained, and rarely perfectly round. Cells in the process of dying when they were fixed pose challenges, as they can look very much like some living cells, specifically neurons. Photoshop is a great resource for this because you can flip between the image showing



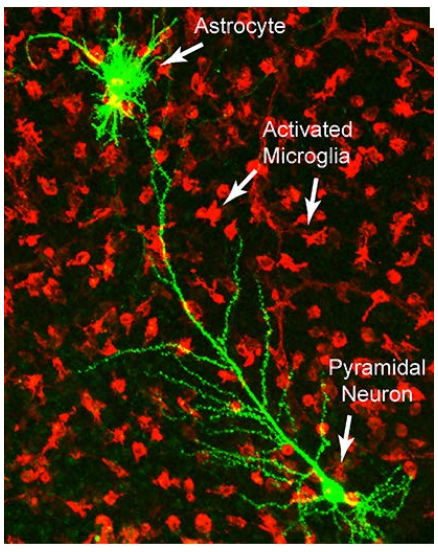


Fig. 2. *Left* – Oligodendrocytes in cell culture stained with O4. The blue spots are DAPI-stained nuclei. This is likely a cluster of several oligodendrocytes. *Right* – Microglia (red) compared to astrocytes and neurons (green) in a rat hippocampal slice culture.

all types of staining and the one showing only DAPI stain to see if the small, round nucleus is associated with other stain which would indicate a living cell, or if it only shows DAPI stain which would indicate it is likely dead (though not always). Be careful to consider cells that are only weakly stained. For example, note in Fig. 3 that there are several DAPI-labeled nuclei that are similar in size to nuclei of GFAP+ astrocytes but apparently lack staining for GFAP. However, if the image is brightened (bottom panel), it becomes apparent that some of the nuclei are associated with weak GFAP staining (white arrows). Thus, to improve identification of all stained cells it is advisable to try different intensities/brightness for a particular channel in Adobe Photoshop to reveal cells which might be only faintly stained.

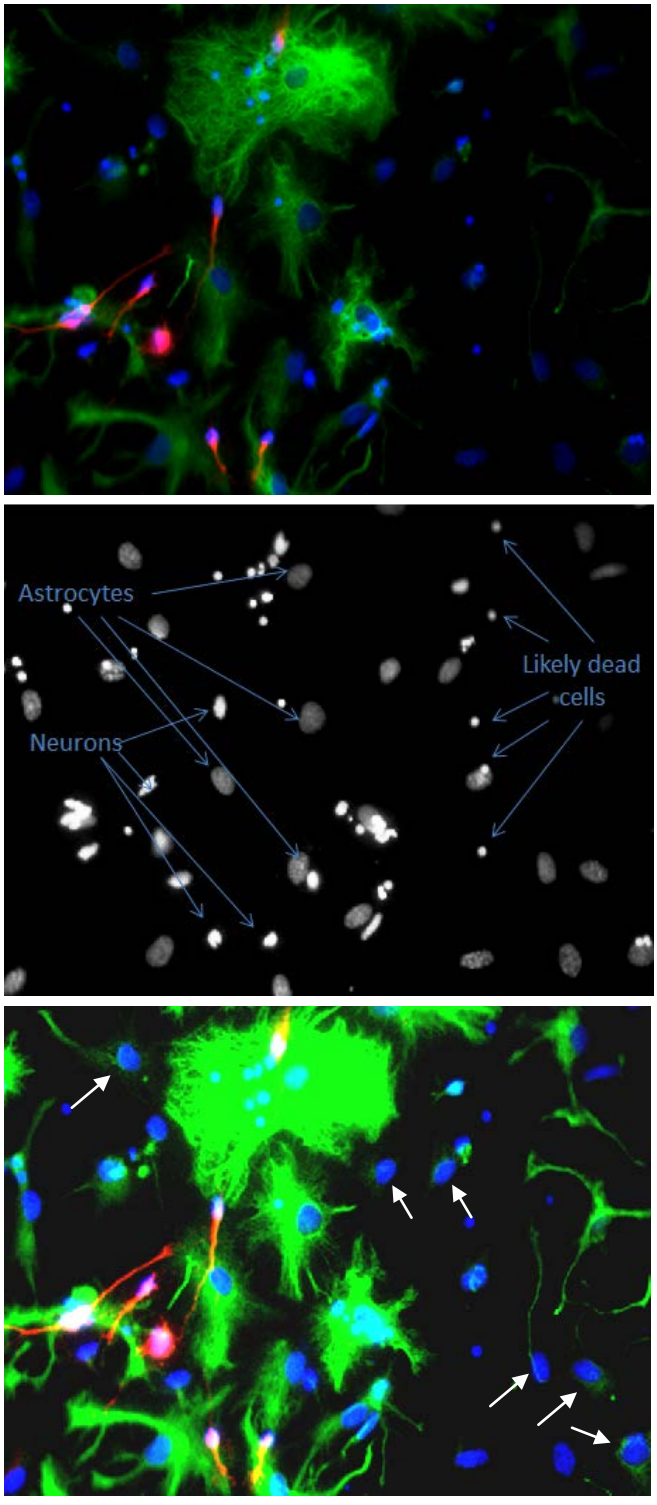


Fig. 3. These pictures are of the same field of view showing different channels of stain. The top picture is a merge of three channels (Green: GFAP; Red: TuJ1, and Blue: DAPI). The middle is the same field of view showing only the DAPI (blue) channel. The bottom is a brighter version of the top image. DAPI labels nuclei of all cell types, whether or not they were dead or alive at the time of fixation. Nuclei of dead cells are usually very small. Note the difference in the size and staining intensity of DAPI-labeled nuclei. It helps to be able to see both picture types to determine which DAPI-labeled structures are associated with stained cells and which show no staining (cells that were likely dead at the time of fixation). There are many examples of dead cells (small, intensely labeled DAPI structures) not pointed out in the middle picture. Notice the difference in size and staining intensity between nuclei of astrocytes and neurons.

Differentiation of cells can be difficult and practice is a great tool. Google and other internet search engines can be helpful as well, but be cautious of the sources of the pictures you're finding. Textbooks are a great reference, as is Dr. Dailey and lecture material.

Document prepared by Jenika Shannon (Neurobiology Lab class of 2010).

Guidelines for Neurobiology Lab Report on Cultured Neuroprogenitor Cells

See the end of this document for the DUE dates.

To solidify your understanding of the cell culture experiments you performed in class, your assignment is to write a lab report (2,500-3,500 words) demonstrating your understanding of the exercise, using data you collected. In preparing your lab report, follow these guidelines:

Abstract (250 word limit) – Provide a short summary of your entire report, including the aim of the study, the basic approach/method, the results, and a conclusion statement. This should be one paragraph.

Introduction (500 word limit) – Write an introductory section (1-2 paragraphs), providing sufficient background to make the goal or intent of the study understandable. At the conclusion of your Introduction, you should make a statement of intent (indicating the purpose or goal of the study) and describe a specific hypothesis or predicted outcome of the study. Your statement of intent could start out something like this: "The goal of this study was…". The Introduction provides you with a good opportunity to convince your instructor that you understand why you have been asked to do the exercise.

Materials and Methods – Summarize the general technical approach, including (a) preparing the cell cultures; (b) control and experimental conditions; (c) antibody staining; (d) methods of data collection; and (e) analysis. For this exercise, you do not need to indicate *all* the details of the protocols, but you must convey that you understand the key elements of the experimental procedure. Note any changes or deviations from the protocols given. Be sure to describe how you identified specific cell types and calculated the density of each cell type in your cultures.

Results – What were the results of the study? Here are specific things you should include:

- *General appearance of cell cultures.* Based on the images you took during the week of culturing, describe (with words and with figures) how your cultures changed from the time you put the cells down until you fixed the cultures. Use representative IMC images to document the changes. Images with figure legends may be embedded in the body of the Results section or presented at the end of the report.
- *Viability of cell cultures.* Based on DAPI staining, calculate the viability (health) of your control and experimental cultures at the time of fixation (number of live- vs. dead-looking DAPI-stained cells). Use the Handout ("*How to Differentiate Different Types of Neural Cells*") posted on the course website to help you identify live vs. dead cell nuclei. Use 3-5 fields of view per condition.
- *Types of cells found.* Describe the cell types - and any interesting patterns - you found in your cultures. Show representative images of each of the cell types you observed.
    - Use Photoshop or similar program to merge fluorescence channels (DAPI and antibody staining). In cases where you performed triple-labeling, show a representative three-color merged image demonstrating the relationships of the different cell types.
- *Fraction of each cell type found in control and experimental conditions*. Analysis should be based on the fraction of 'live' cells. For each condition, report the raw numbers (i.e. how many cells did you count, in how many images, from how many coverslips?), as well as any calculated values. Analyze 3-5 fields of view per condition. Your data should be presented in a table, which could look something like this:

(Sample) Table 1: Fraction of Cells by Cell Type									
	# of 'dead' cells	# of 'live' cells	Total # of DAPI nuclei (live+dead)	# of GFAP+ cells	% live cells that were GFAP+	# of TuJ1+ cells	% live cells that were TuJ1+	# of O4+ cells	% live cells that were O4+
Control									
Coverslip 1									
Field 1									
Field 2									
Field 3									
Coverslip 2									
Field 1									
Etc.									
Experimental									
Coverslip 1									
Etc.									

- It may be helpful to include *graphical* representations (e.g., bar graphs) to summarize your data.
- *Test of hypothesis.* Perform an analysis of the data to determine whether there are statistically significant differences in the types of cells found in the control and experimental conditions. (You will need to rely on your acquired knowledge of Biostatistics.) Was there sufficient data to perform appropriate statistical analyses?

Discussion (1,000 word limit) – Discuss how your results relate to the specific question(s) and goals of the study, as stated in your Introduction. What did you expect to find, and why? Did the data analysis support your hypothesis or prediction? How might you explain any unexpected results? Discuss shortcomings of the study and suggest possible solutions or future studies.

Figures and Figure Legends

- Show representative data in numbered figures and include figure legends. Cite the figures in order in the Results section.

Literature Cited – This section includes the full citations for any references (including primary literature articles, textbooks, laboratory handouts, lectures, etc.) you have cited in your report.

** For more information on how to write a lab report, see the document, "Components of a Research Report," posted on the course website.*

**** DUE Dates ****

- **Friday, March 9, 2012 (5pm) – Submit your hypothesis, with a brief explanation of your reasoning.**
- **Thursday, March 22, 2012 (5pm) – Submit a table of cell counts.**
- **Thursday, March 29, 2012 (5pm) – Submit your completed lab report.**

You may provide a hard copy of your materials to Prof. Dailey (369BB), or email an electronic copy (Michael-e-dailey@uiowa.edu), by the deadline. Receipt of your electronic copy will be acknowledged with a return email. For the final lab report, if you send an electronic version, please also submit a paper copy within two working days after the deadline.