

## ARTICLE

# Every Cell Counts: An Inquiry-Based Approach to Address a Novel Research Question in an Undergraduate Neuroscience Lab

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A science-based curriculum that encourages hands-on experiences, skill development, and promotes student engagement are critical components in both successful undergraduate psychology and neuroscience programs. This lab explored an inquiry-based research project focused on microscopy skills, critical thinking, and independent research design. This lesson used a novel research question (*How many serotonergic cells are*

*located in the dorsal raphe nucleus?*) to engage students in research and methodology design. The resulting lab received positive feedback from students and provided data about the serotonergic system in a previously unreported species.

**Key words:** *inquiry-based instruction; neuroanatomy; microscopy; serotonin*

Current consensus among leaders in the design of undergraduate education in psychology promotes a strong foundation including a “science-based curriculum that requires students to demonstrate skills and behaviors of scientists” (p.655, Dunn et al., 2007). Furthermore, the current American Psychological Association guidelines for the undergraduate psychology major advocate theory and research in the domain of biological bases of behavior, including physiology and comparative assessments (American Psychological Association, 2007). These recommendations can be readily applied to undergraduate programs in neuroscience as well. With these recommendations in mind, an activity was designed for our laboratory in physiological psychology to challenge students to think critically, engage in independent research design, and to apply microscopy skills within the context of addressing a novel research question. An additional goal of this lab exercise was to develop an experience grounded in inquiry-based instruction as an alternative to traditional laboratory instruction (for a brief review of laboratory instruction styles, please see Domin, 1999).

Inquiry-based instruction is generally characterized by student designed and driven projects without a prescribed outcome (Domin, 1999). It has been suggested that this style of instruction may result in more positive student attitudes about science education (Domin, 1999). In addition, I sought to design a project that would not require purchasing additional equipment or consumable supplies and would utilize techniques common to undergraduate labs in physiological psychology or related courses (prepared slides, microscopes, desk top computers). The software used in this lab is freely available open-source software which can be downloaded from a website maintained by the National Institutes of Health (NIH; ImageJ software). This software is an economical and useful resource for student projects.

This inquiry-based lab activity utilized Concannon and Brown's (2008) four steps to incorporating inquiry; (1) engage students in driving questions (*students were asked “How many cells are there? How can you determine*

*this?”*); (2) allow students to create a strategy to explore their predictions (*students were directed to design their own research strategy and predictions*); (3) provide materials and time required to perform the investigation (*students had two weeks to conduct their research, and one week to write a follow-up lab report; all materials were provided in lab*); and (4) encourage students to reflect on their results to guide future explorations (*students reflected on their research by sharing their data in class, and writing lab reports which included suggestions for future research*).

In this project, students were challenged to estimate the number of serotonergic cells present in the dorsal raphe nucleus (DRN) of the gerbil. Distributions of serotonergic cell bodies have been described for many species but have not been widely quantified (fish: Kah & Chambolle, 1983, Yamanaka et al., 1990; newt: Fasolo et al., 1986; turtle: Ueda et al., 1983; frog: Ueda et al., 1984; pigeon: Challet et al., 1996; rat and cat: Takeuchi et al., 1982; Highveld gerbil: Moon et al., 2007; Mongolian gerbil: Janusonis et al., 2003). Although quantitative serotonergic cell counts for the DRN are available for mice (mean  $\pm$  standard deviation serotonergic cell counts; 7178  $\pm$  195, Takeuchi et al., 1992; 9180  $\pm$  390, Ishimura et al., 1988, 1989), they are not currently published for the Mongolian gerbil (*Meriones unguiculatus*).

Within the context of comparative neuroanatomy, the gerbil provides an interesting example of a crepuscular rodent, whose behaviors and neuroanatomy may be different from those of other mammalian models (Pietrewicz et al., 1982). The DRN contains the majority of serotonergic cell bodies within the central nervous system, provides the primary source of serotonergic innervation to the forebrain and is highly conserved across many species, (Halliday et al., 1995; Jacobs and Azmitia, 1992). In gerbils, the DRN receives afferent projections from the retinas (Fite et al., 1999, 2003) and terminates efferent projections in nuclei associated with circadian rhythms, including the intergeniculate leaflet and the suprachiasmatic nucleus (Glass et al., 2000; Meyer-Bernstein and Morin, 1999). Examining the comparative

neuroanatomy of the nucleus in this species is of particular interest because of its unique involvement in circadian changes in the serotonin system and central nervous system functions (Birkett and Fite, 2005).

## MATERIALS AND METHODS

Twenty-three students enrolled in an upper division psychology lab course at a midsized, regional state university, completed this activity. In this class, over 90% of students were declared psychology majors (n=21 students). In addition, the class included one biology major and one graduate student. Of the undergraduate students, 36.4% were seniors (n=8), 50% were juniors (n=11) and 13.6% were sophomores (n=3). Students worked in eight pairs or small groups to complete the project.

During the first two-hour lab session, students were introduced to basic microscopy techniques and research methods, including principles of animal research, tissue and slide preparation and immunohistochemistry. The instructors for the course demonstrated microscopy techniques and assisted groups in working with the microscopes to adjust magnification, focus, etc. To practice working with microscopes, the students viewed a tutorial video and worked through several basic microscopy activities (see *additional materials* section). Students were then presented with their research challenge: to estimate how many serotonin-containing cells are present in the midbrain (DRN) of a gerbil.

In the second two-hour lab session (one week later), students were given access to sets of previously stained slides, prepared to include the DRN of a gerbil. Processes used to stain the tissue and prepare the slides as well as the thickness of all tissue sections and the procedures of staining alternate sections were discussed in class prior to beginning the lab activity. The slides for this experiment were previously stained using an immunohistochemical technique and were donated to the teaching lab (gift of Dr. Katherine Fite; University of Massachusetts Amherst). Tissue was sectioned on the coronal plane throughout the mesencephalon on a freezing microtome in serial in 40 $\mu$ m thick sections, with alternate sections mounted onto gelatin-coated, cover slipped slides. Serotonin immunostaining was based upon a modification described by Janusonis et al. (1999) and Birkett and Fite (2005). For a graduate level or more advanced course, a semester long project might include tissue preparation, staining and slide making. If access to other stained tissue (various species, brain regions, for example) is available, the lesson could be adjusted accordingly.

Students had access to light microscopes (Fisher Scientific monocular microscopes with 4, 10, 40x objective lenses; Boreal B1-220 binocular microscope with 4, 10, 40x objective lenses) and a digital camera (Motic Moticam 483). Digital images were captured using Motic Images Plus 2.0 ML software and analyzed using ImageJ software (NIH ImageJ; <http://rsbweb.nih.gov/ij/>) on a desktop computer (Dell Desktop computer, Pentium 4 processor). For a brief overview of basic imaging capabilities of ImageJ for microscopy (including an automated cell counting protocol), please see Collins (2007) and Papadopoulos et al.

(2007). The students had access to manual counting techniques used with the light microscopes, and automated cell counts from digital images of their stained sections. Each group of students working together selected one representative set of slides from one gerbil to quantify the number of serotonin-stained cells in the DRN. Students were instructed to design their own methodology to most accurately quantify the number of serotonin-stained cells present in their representative subject. Instructors were available throughout the lab to help students design their experimental methodology.

## RESULTS

The student research groups estimated cell counts of serotonin-containing cells in the gerbil DRN ranging from 2208 to 8720 cells with a mean of 5983 and standard deviation of 2316.5 cells (see Table 1). The students used a range of methodologies to achieve their final estimates. The students were encouraged to design their own methodology and encouraged to review primary literature and resources outside of class.

The approaches that students took to counting the cells varied widely. In general, the groups choose to either (1) view the slides under light microscope and count cells visually or (2) capture images of the slides using the digital camera, print out the images and count the cells in the pictures. Although they had access to an automated cell counting software, students did not prefer this approach. Providing clearer directions and optimizing the parameters for the automated cell counting may lead to more use in the future. Some students chose to count cells in quadrants under high magnification with a smaller field of view, while other students used a lower magnification in order to better view the entire region of interest. All students attempted to account for cell estimates in unstained alternate sections. Some groups chose to average counts within a single slide, or in every other slice. Several groups chose to estimate based on representative slides or slices. This approach allowed them to account for poorly stained or damaged tissue slices they encountered. Two groups counted manually using light microscopes, adopting a between-students approach, later correcting for inter-rater reliability.

Group Number	Estimated Cell Count
1	5622
2	6816
3	2208
4	3915
5	7383
6	4600
7	8270
8	8600
Mean $\pm$ SD	<b>5983 <math>\pm</math> 2316.52468</b>

Table 1. Student-generated cell counts. These cases represent the eight group projects in the class.

Throughout this experience, students were guided through feedback and discussion with both the instructor

and teaching assistant in the lab. This feedback was directed at helping the students to design their methodology and guide their data collection. At the conclusion of the lab, all of the student groups shared their methodology with the class and received feedback from other students on their techniques. As a class, we also reviewed the literature concerning cell counts in this region of the brain and discussed the potential accuracy of our counts. This was an important feature of the activity and allowed students to assess the reliability of their own cell counts. Overall, most students produced cell count data that was within a possible range for this species based on the data available from mouse research.

The preliminary data presented here estimating serotonergic cell counts in the gerbil DRN (Table 1.) suggest that they may be consistent with similar counts in the mouse (Ishimura et al., 1988, 1989; Takeuchi et al., 1992), but may be an underestimation of cell counts in the gerbil. As a larger species with an increased neocortex and increased number of efferent projects from the DRN, the number of serotonergic cells for the gerbil DRN may be better reflected by the cell counts at the higher end of the range presented here (8270, 8600 cells). This apparent underestimation may have been the result of inaccuracies in cell counting technique and the unfamiliarity of the students with the structure of the DRN across its five levels. At the conclusion of this lab, the class discussed the significance of their contributions to this body of literature. The students suggested that this research should control for inter-rater reliability, would require replication prior to reporting and should be subject to peer review before drawing any additional conclusions.

## DISCUSSION

This lab used a novel research question to promote student engagement and development of important research skills (microscopy and independent research design).

In general, students responded positively to this research project. In a post-lab assessment, students were asked to anonymously respond to questions about their experience in lab on a scale of 1 (*strongly agree*) to 5 (*strongly disagree*). This assessment was based on a modification of the laboratory survey by Mead (2008). Results of this assessment are presented in Table 2. In particular, students indicated that this lab “required me to use problem-solving techniques” and “this class required me to think critically about a problem.”

Positive student comments on the assessment about this exercise included “The experience was a good learning process,” “I thought it was an interesting topic to work with; I enjoyed the experience,” “...our method was imaginative and accurate,” and “Overall great experience!”

At the conclusion of this lab, students submitted a lab report including a rationale and implications of their research. Lab reports were graded based on a rubric that was available to all students throughout the lab activity (see *additional materials*). Notable aspects of these reports included student-generated data tables and graphs of cell counts, suggestions that this line of research may be

valuable in studying the evolution of the serotonergic system across mammals (when compared to data available for mice, rats and cats), using the gerbil serotonergic system as a model for screening drugs that may affect the activity of the serotonin system, and the need for replication of these results to draw accurate conclusions.

Questions	Response (mean ± SD)
This lab helped me to understand microscope techniques.	3.90 ± 1.09
This lab helped me to understand some staining techniques used to visualize cells.	3.4 ± 1.19
This lab helped me to learn about neuroanatomy (structure of the brain).	3.43 ± 0.81
This lab required me to use problem-solving techniques.	4.48 ± 0.68
This lab required me to do independent research (looking up information outside of class).	3.14 ± 1.39
This class required me to think critically about a problem (estimating cell counts).	4.38 ± 0.80
This lab was interesting.	3.57 ± 1.12
This lab increased my interest in the class.	3.48 ± 1.17
I enjoyed this lab experience.	3.52 ± 1.03
I would recommend this lab to other students interested in the topic.	3.52 ± 1.12
<b>Overall</b>	<b>3.68 ± 1.04</b>

Table 2. Student responses to assessment questions.

Creating an engaging lab environment and promoting student-designed research may promote class attendance and student success. Moore (2008) concluded that lab attendance is “strongly correlated with students’ academic performance in introductory science courses” (p. 69). Applied to an upper division course, this type of lab activity may facilitate student success in corresponding lecture coursework as well.

Incorporation of inquiry-based instruction into undergraduate science courses has not been thoroughly reviewed. However, in one recent case study, Park Rogers and Abell (2008) identified key goals of instructors in inquiry-based curricula for “having students learn how science is done” and “developing students’ content knowledge through big ideas shared across the science disciplines” (p. 595-596). The researchers noted that students developed a “community of learning” during the experience in which “they recognized that science is not something that is individually practiced, but is a team effort” (p. 603). These goals and the social nature of scientific problem solving were evident in the present lab as students collaborated on the design of their methods and interpretation of their results. As an inquiry-based approach to a lab, this activity was useful in promoting problem solving and critical-thinking, as indicated through student responses to the lab.

## FUTURE DIRECTIONS

Areas of improvement for this lab include incorporating additional neuroanatomy and neurochemistry content information and transitioning from a “partial” level of inquiry in which the research question was provided to the

students, but students were responsible for constructing the methodology and solution, to a more fully developed level of inquiry in which students also construct the specific research question of interest (for a suggested rubric of level of inquiry, see Fay and Lowery Bretz, 2008). In this pilot lab, the exercise was offered early in the term before subsequent units that included additional information about the serotonin system and neuroanatomy. Providing this activity after these units may be more integrative and could provide students with more opportunities to make connections across various topics. Additionally, this lab could be expanded to include preliminary data analysis and descriptive statistics of the cell counts to incorporate applied practice using these techniques.

Methodologically, it would be valuable to have student groups discuss the accuracy of various methods or to have groups use and try to replicate the methodology and counts of other groups. Another option might be for the class to discuss the methodology designed by each of the groups and choose one method for all groups, comparing counts for a given approach. Providing students with access to figures of the different levels of the DRN would further assist them in their methodological design. Clear figures of the five levels of the DRN can be found in Janusonis et al. (1999) (see Figure 2). This figure can be used as a guide to identify levels of the DRN as well as landmarks such as the cerebral aqueduct and the medial longitudinal fasciculus. Clearly delineating the boundaries of the DRN may help in the future. This aspect of the activity may not have been clear to students and may have contributed to the wide range of cell counts.

In the future, improvements to assessing the use of this activity should include quantifying outcomes through a more formal assessment of knowledge. This might include a pre- and post-test comparison of content knowledge or a more formal reflection assignment to accompany the lab. Additional assessment should also include skills development. Microscopy technique, writing skills, research design methodology or research to prepare a more formal literature review for a lab report could be included in a skills assessment.

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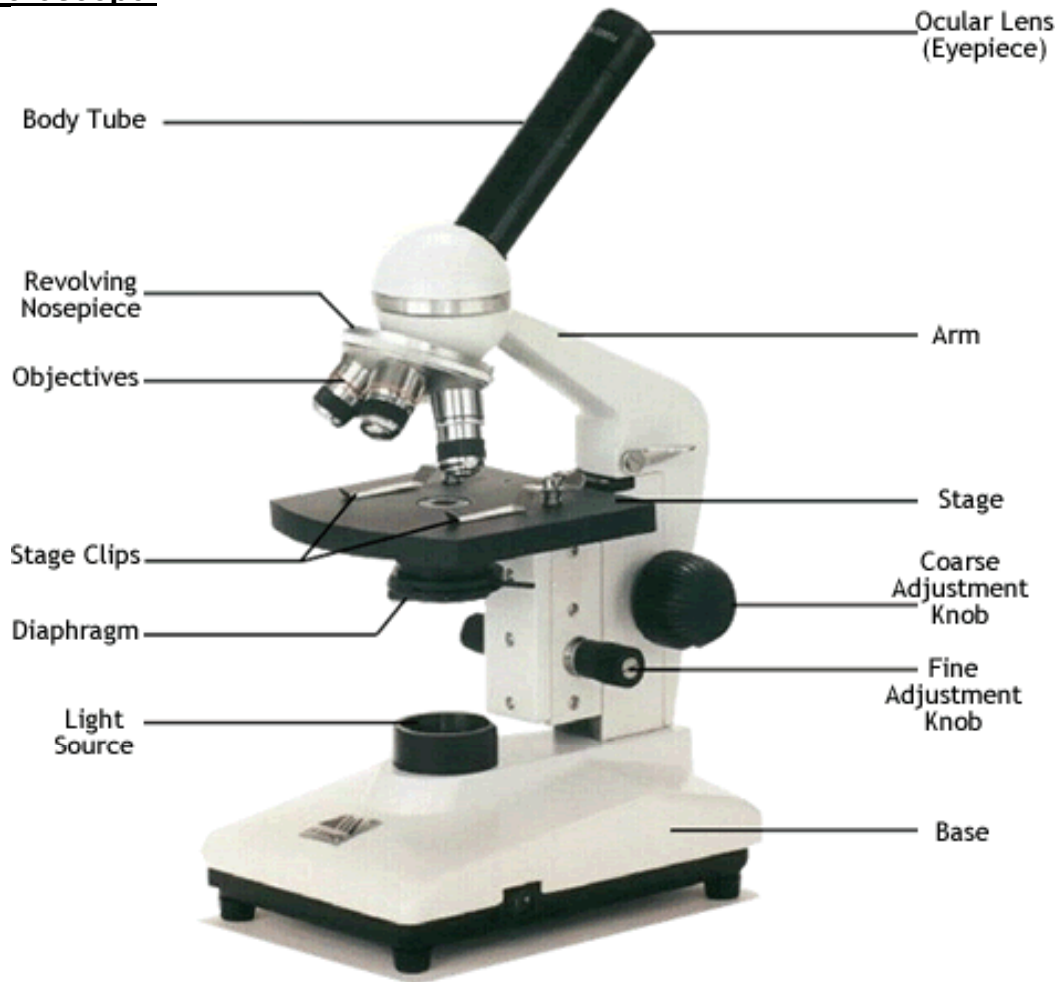


## ADDITIONAL MATERIALS

### Microscopy Lab

#### Part 1

#### Parts of a microscope.



#### Microscope use demo:

- View tutorial: <http://www.udel.edu/biology/ketcham/microscope/joelle.mov>
- Virtual Microscope: <http://www.udel.edu/biology/ketcham/microscope/scope.html>
- Parts
- Handling
- Focusing
- Switching objectives
- Handling slides

**Magnification Calculation**

What is the magnification power of the low power objective?

The medium power objective?

The high power objective?

What is the magnification power of the ocular lens?

If you view a slide using the 10x **objective** and the **ocular** lenses, how many times is it **really** magnified?

What is a formula for total magnification under a light microscope?

**Measuring with a Microscope**

Use a ruler to determine the width of the viewing field under the scanning objective. Position the ruler so that the millimeter marks are visible in your viewing field. Remember that there are 1000 micrometers in a millimeter.

Estimate the length (diameter) of your viewing field in micrometers \_\_\_\_\_

You cannot use this method to determine the diameter under high power (try switching objectives). Instead you can use a mathematical proportion method to determine the diameter under high power.

High power field diameter = low power field diameter x low power magnification / high power magnification

What is the diameter (in micrometers) of your high power field \_\_\_\_\_

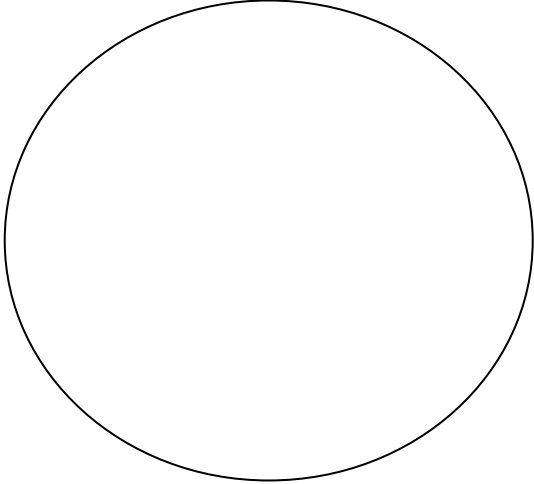
Fill out the table below after viewing various specimens.

Name of Object	Measurement of Object

**Letter “e” activity.**

The letter “e” is one way to familiarize yourself with how images are seen through a microscope. Draw or cut out a letter “e”. View the “e” under low and medium power under the microscope.

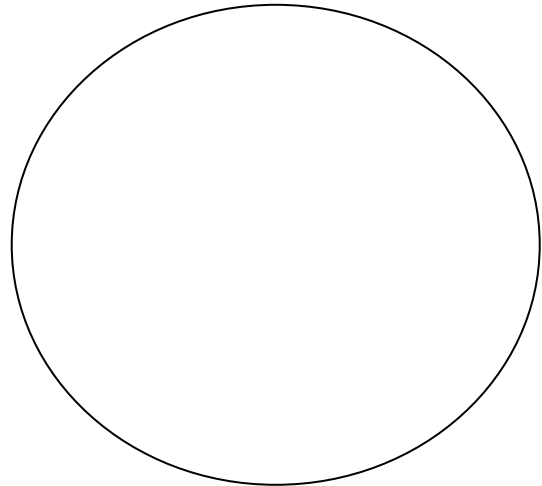
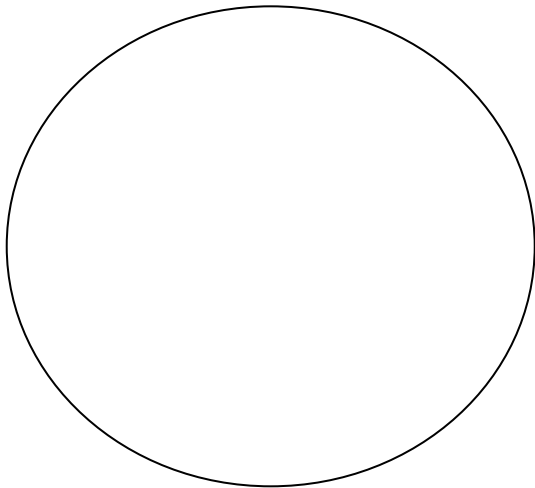
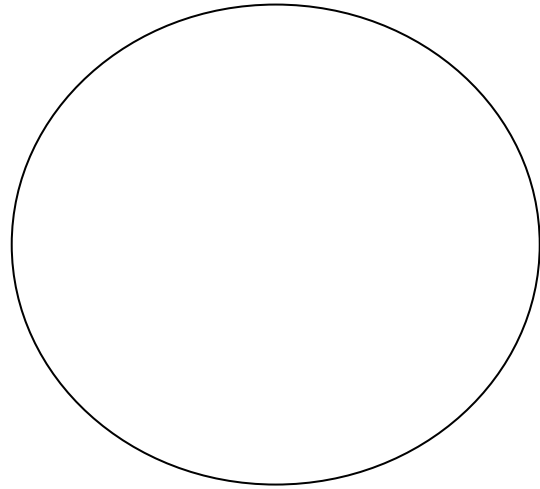
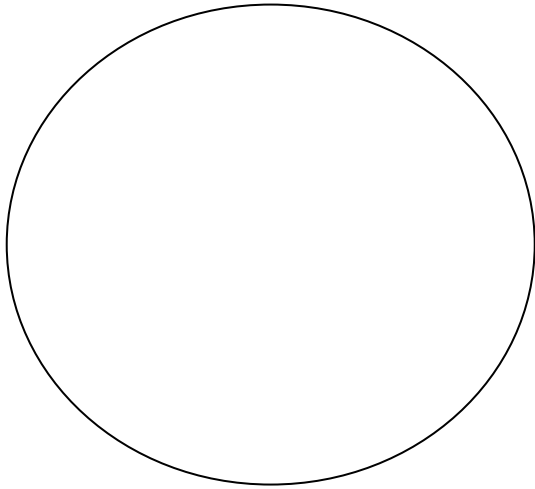
Sketch and describe what you see.



What do you conclude about the optics of the microscope?



Find 4 ***different*** stained neurons in some of the prepared slides. View it under medium magnification. Sketch what you see and label as many parts of the neuron as you are able. ***Label your specimens.***



Practice using the ImageJ imaging software in the microscope lab. Take one picture of a neuron. Make sure your picture is optimized! Find the best magnification, the best focus, the best centering. Capture your image and print it out. You may need to email the image to yourself and print from another computer. Each person should produce a unique image!

## Part 2

As a researcher you are interested in examining serotonergic midbrain structures across mammals. You feel that this research may help you to understand the evolution of the serotonin system in mammals. You are currently investigating the gerbil brain.

**Research Question:** Estimate how many serotonin-containing neurons are present in the midbrain of a gerbil.

Select a subject to study. Within your group discuss and determine the best way to estimate the number of cells present. You can use any of the tools available to you in the lab, including cell counting and imaging software. ***Please be aware that other groups may need to share equipment with you.*** Once you come up with your research plan, you will need to work efficiently and considerately in order for everyone to conduct his or her research. ***Please demonstrate the utmost care and respect in working with this equipment and tissue samples.***

**Some (possibly) relevant information for your consideration:**

- These slides contain alternate sections of tissue (meaning that one “slice” is missing between each tissue sample)
- These tissue samples were sliced on a sliding, freezing microtome and mounted on slides before being coverslipped.
- To visualize the serotonin-containing cells, a serotonin anti-body was used in an immunohistochemical staining procedure with the tissue.
- Each slice of tissue is approximately 40 micrometers thick.
- The sections mounted on the slides are in approximately the correct rostral to caudal order.

**Remember to record your data and your methodology in your lab notebook. You will need this information to include in your lab report. Be as specific as possible!**

Guidelines for the lab report:

1. Each individual will submit a lab report. You may work with your lab group, but all reports must be original writing.
2. The report should be approximately 3 pages, double-spaced, proof-read, spell-checked and should follow APA formatting guidelines.
3. Reports should include:
  - a. A descriptive **Title** on a cover page
  - b. Your **Research Question** and any hypotheses you had prior to the experiment
  - c. An **Introduction Section**
    - i. Including rationale for your research
    - ii. Background information your reader would need to understand the experiment and why you did it
  - d. A **Methods Sections**
    - i. Including subject/specimen details
    - ii. All methodology and research design details (sufficient that someone could replicate your experiment if they read your report)
  - e. A **Results Section**
    - i. Including all of your data, data tables, graphs, and descriptions of the results of the experiment
  - f. A **Conclusions Section**
    - i. Including your interpretation of the results and data and their implications (*What can you do with this information? What would the next step in the research be?*)
  - g. A **Reference Section** (including any sources you may have consulted)
  - h. Attach the pages from these lab handouts to the report.

Lab Portion	Criteria and Qualities	Approaches Expectations	Meets Expectations	Excellent/Exceeds Expectations	Point Value
Applied Lab Activities and Exploration 5 Points Possible	<b>Magnification Calculation</b>	Activity was not completed or student failed to demonstrate basic understanding of microscope magnification. <b>(0 pt.)</b>	Student demonstrated inconsistent understanding of microscopy or the activity was incomplete. <b>(1/2 pt.)</b>	Student demonstrated basic understanding of microscopy and the activity was complete. <b>(1 pt.)</b>	<b>0-1</b>
	<b>Measuring with a Microscope</b>	Activity was not completed or student failed to attempt to measure 4 different specimens. <b>(0 pt.)</b>	Student attempted to measure specimens but did not complete and/or demonstrate comprehension of the activity. <b>(1/2 pt.)</b>	Student completed the activity and succeeded in measuring 4 different specimens. <b>(1 pt.)</b>	<b>0-1</b>
	<b>Letter “e” and Neuron Observation</b>	Activity was not completed or the student failed to make an effort to observe prepared slides or letter “e” under microscope. <b>(0 pt.)</b>	Student attempted to complete activity but failed to complete all observations or observe “e” under microscope. <b>(1-2 pts.)</b>	Student thoughtfully completed the “e” activity as well as all 4 prepared slide observations. <b>(3 pts.)</b>	<b>0-3</b>
Lab Report 20 Points Possible	<b>Research Lab Report: Basic Criteria (e.g. length, format, effort)</b>	Lab report was missing components, failed to meet minimum requirements, or was not edited <b>(0-2 pts.)</b>	Lab report was missing only minor components, writing is generally clear, basic criteria are met, and errors may be evident in writing. <b>(3-4 pts.)</b>	Lab report is complete with all basic criteria met with an obvious effort made to use appropriate editing and thoroughness. <b>(5 pts.)</b>	<b>0-5</b>
	<b>Research Lab Report: Exploring the Research Question</b>	Lab report failed to contain a descriptive title, research question, and/or a rationale for research. Little effort was made to explore the research question. <b>(0 pts.)</b>	Lab report was a component. or a lack of thoughtfulness was made to explore the research question. <b>(1 pt.)</b>	Lab report contained a descriptive title, research question, and a thoughtful effort to explore rationale for research. <b>(2 pts.)</b>	<b>0-2</b>
	<b>Research Lab Report: Specimen, Methodology, Research Design</b>	Lab report failed to contain description of subject, methodology, or research design. If the reader wanted to repeat your methods they would be unable because details were missing. <b>(0-3 pts.)</b>	Lab report did not include a thorough explanation of the research components regarding the subject, methodology, and/or research design. <b>(4-6 pts.)</b>	Lab report contained a thorough explanation with all the necessary components needed to repeat the research methods. <b>(7-8 pts.)</b>	<b>0-8</b>
	<b>Research Lab Report: Results, Conclusions, Implications, and References</b>	Lab report failed to contain the results, conclusion, implications or references <b>(0-2 pts.)</b>	Lab report lacked references when necessary or a thorough explanation of the results, conclusion, or implications. Lab report lacked information, data, or methodology to support your results or conclusions. <b>(3-4 pts.)</b>	Lab report contained a thorough explanation of your results and conclusions. Report discussed the implications of the research thoughtfully, referenced sources as needed, and included a descriptive conclusion including suggestions for future research <b>(5 pts.)</b>	<b>0-5</b>