Overview

Plenty of Planaria Student Background

Planaria are freshwater flatworms. Although they seem simple, they are actual quite COMPLEX! They have muscles that they use to move, assisted by cilia on their underside. They have a digestive system, but it is incomplete (partially open). While they lack circulatory and respiratory systems, they do possess a specialized excretory system.

Planaria have a rudimentary 'brain' consisting of two groups of neurons (ganglions) located in the anterior (front) end. Two nerve cords run along the side towards the back of the animal, giving the nervous system a ladder appearance. This system gives planaria the ability to have varied behaviors. Planaria have the ability to respond to their environment by moving towards or away from stimuli. A positive and negative response to environmental cues is called a 'taxis'. So, moving towards light is called 'positive phototaxis'.

Planaria have a special capability. They are famous for being able to regenerate parts of themselves! In this lab, we will use them as a MODEL ORGANISM for understanding the REGENERATION process and the cells involved. Only one type of cell in a planaria—the 'neoblast'—is capable of dividing. It must, therefore, be able to differentiate into any type of complex tissue the planaria requires for regeneration.

During this investigation you will conduct an experiment to learn more about the ability of planaria to regenerate.

Whenever scientists use animals, they need to carefully consider the ethical and legal guidelines in addition to the benefits that the research may provide. In your proposal, you will need to address ethical guidelines.

You will have three planaria per team

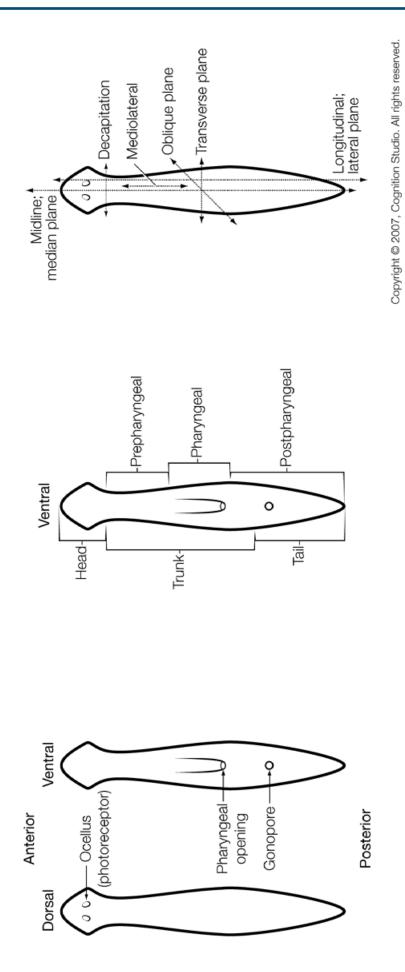
- 1) Two "experimental" planaria
 - a. Experimental planaria #1 will be cut into half, with a front (anterior) and rear section (posterior). Every team in the class will do the same! (Why is it important to have experiments repeated in order to generate more data?)
 - b. Experimental planaria #2 will be cut in a manner determined by your team.
- 2) A "control" planaria which will not be cut

Review the Planaria anatomy on the other side of this sheet, then complete the **Research Proposal Form** with your team and receive approval from your teacher before proceeding.

Student Handout 1

Plenty of Planaria

Student Background



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Animals in Research

When animals are used in biomedical research, laws, regulations and guidelines govern their care.

These include requirements that:

- procedures involving animals be relevant to human or animal health
- the minimum number of animals be used to obtain valid results
- alternatives to animals be considered
- animal pain or distress be avoided or minimized
- living conditions for animals be appropriate for their species
- research scientists and those caring for the animals be properly trained and qualified

These requirements are sometimes summarized as the **3R's**:

- 1. Replacement-using other models when appropriate
- 2. Reduction-using the minimum number of animals necessary
- 3. **Refinement**-enhancing animal welfare and ensuring the best conditions possible, minimizing pain and distress

Groups that review research involving animals ('Institutional Animal Care and Use Committees'- IACUCs) suggest ways to minimize pain and distress, and work directly with researchers before experiments have started. The IACUCs use the 3R's as principles that underlie the humane treatment of animals in biological research. A fourth R – Respect for the organism– is often added. These requirements are based on the idea that good science evolves with, and as a result of, humane science.

Background on laws:

Animal Welfare Act (AWA)

The Animal Welfare Act sets federal standards for all aspects of care for laboratory animals. It was enacted into law in 1966 and has been amended by the U.S. Congress several times. The act applies to all public and private research facilities in this country. Facilities must be registered by the US Department of Agriculture and comply with their regulations, including unannounced annual inspections. Also, all facilities must establish an Institutional Animal Care and Use Committee (IACUC). The committee ensures that applicable federal, state, and local laws and regulations are met, reviews and approves procedures involving animals before they take place, and inspects facilities twice a year for compliance with the AWA.

Health Research Extension Act.

This 1985 federal law applies to facilities that receive funding to do research from the federal government, in contrast to the Animal Welfare Act, which applies to all facilities regardless of the source of funds. The legal and regulatory requirements of the act are very similar to those of the Animal Welfare Act, and they apply to all research supported by the U.S. Public Health Service (PHS) involving vertebrate animals, including rats, mice and birds, which are not covered under the AWA.

http://www.nwabr.org/research/regulations.html

http://caat.jhsph.edu/programs/workshops/20th/locke.htm

Name

Using the 3 R's in Animal Research

Replacement – Please indicate if *alternative procedures* (that do not require animals) exist that might meet the project's needs. If alternative procedures exist, please explain why you feel that animals must still be used:

Reduction - Please provide an explanation why you feel that the number of these animals to be used on the project represents the *minimum number required*:

Refinement - Please explain the methods and techniques that will be used to *minimize distress to these animals.*

Investigator Certification:

We certify that we will adhere to the guidelines contained in this proposal, and will not deviate from any of the procedures contained unless they are formally approved by the Institutional Animal Care and Use Committee (IACUC). We certify that the studies performed under this project are not unnecessarily duplicating research that has already been done before, that all personnel working on this project are appropriately trained in a manner approved by the IACUC, and that the scientific requirements of the project and the welfare of the animals used for the project will be maintained.

Signed

Date

Plenty of Planaria

Research Proposal Form

Name ____

Team Name

Team Members

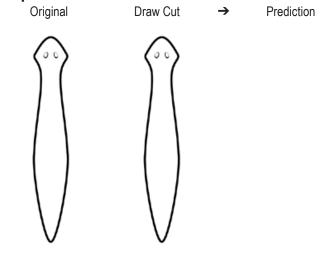
Project Proposal Title

Please summarize the purpose and goals of the project.

What species are you using? How many animals are you using?

PROCEDURES Diagram your cuts, and your expected results

Experimental Planaria #1



What hypothesis are you testing with Planaria #1?

If your hypothesis is supported, what do you predict you will see?

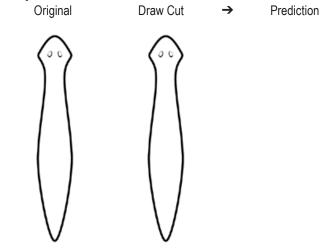
What is your 'manipulated' (independent variable)?

What is your 'responding' (dependent variable)?

What measurements could you make?

PROCEDURES Diagram your cuts, and your expected results

Experimental Planaria #2



What hypothesis are you testing with Planaria #2?

If your hypothesis is supported, what do you predict you will see?

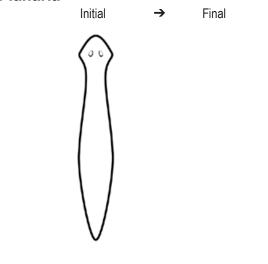
What is your 'manipulated' (independent variable)?

What is your 'responding' (dependent variable)?

What measurements could you make?

PROCEDURES Diagram your control, and your expected results

Control Planaria



Date _____

_ Period __

Plenty of Planaria

Investigation

Collect the following materials.

Observe a planaria and sketch it.

Create your cuts and collect your initial data.

Materials (per group)

- small petri dish containing 3 Planaria
- microscope slides
- lens paper
- 1 scalpel
- 1 pipet
- camel's hair brush or small paintbrush
- dissecting microscope or magnifying glass
- wax pencil or sharpie
- ruler (clear)

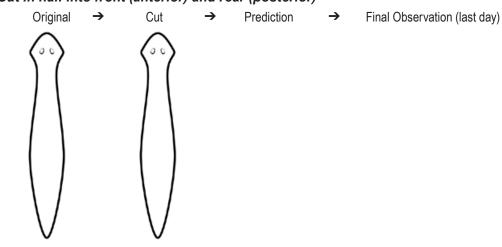
Procedure

- Using a pipet, put a planaria on a microscope slide with a drop of water (if the planaria gets stuck in the pipet, flush out using water).
- Observe the planaria under the dissecting microscope or with a magnifying glass.
- 3) Sketch a planaria and label the following structural components
 - head
 - tailphotoreceptors (eye spots)
 - photorect
 pharynx
- 4) Label your petri dish with your name and group.
- 5) Wrap a piece of lens paper around a second microscope slide. This will form a cutting surface.
- 6) Using the pipet and/or camels hair brush, place experimental planaria #1 on the microscope slide that is wrapped in lens paper. Allow the planaria to become fully extended on the slide.
- 7) Put the slide under the microscope or magnifying glass.
- 8) Use the scalpel to make your cut for experimental planaria #1.
- 9) Measure and record the length of the front (anterior) and the rear (posterior) pieces.
- 10) Gently place the separated or cut planaria back in the Petri dish using the pipet or camels hair brush.
- 11) Repeat numbers 6 through 11 for experimental planaria #2, making cuts and measurements according to your group's plan.
- 12) Measure the length of your control planaria without making any cuts. Gently place it back in the Petri dish.
- 13) Make sure there is water in the Petri dish. Cover the Petri dish and place it in a shady area at room temperature.
- 14) Clean up your lab area and return all materials

SKETCH:

Experimental Planaria #1

Cut in half into front (anterior) and rear (posterior)



Record the length of the front (anterior) section just AFTER you cut. This is your INITIAL front length: _____ Record the length of the rear (posterior) section just AFTER you cut. This is your INITIAL rear length: _____

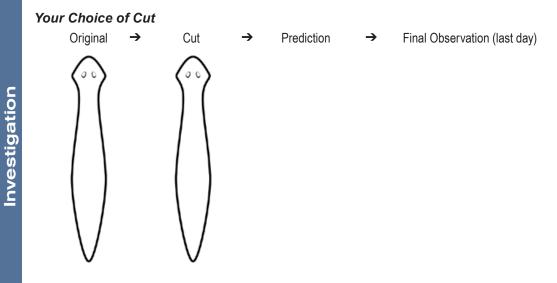
Date	Sketch	Front/Rear Length(s)	Behavioral	Other
Initial				

Record the FINAL length of the front (anterior) section:

Record the FINAL length of the rear (posterior) section: _____



Experimental Planaria #2



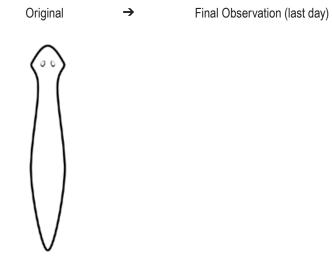
- Record your team's measurement of choice just AFTER you cut. This is your INITIAL length:
- If your team has more than one segment to measure, record the second measurement just AFTER you cut.

Date	Sketch	Measurement of Choice Length(s)	Behavioral	Other
Initial				

Record the FINAL measurement of choice for your team's cut: _

If your team has more than one segment to measure, record the second FINAL measurement of choice.

Control Planaria



Record the INITIAL length: _____

Date	Sketch	Length(s)	Behavioral	Other
Initial				

Record the FINAL length: ______

Planaria Data Analysis – Team Project

1. Calculate the % change in length for experimental planaria #1 that was cut in half, for each piece. Show your work below. *Express your answer as a percent.*

% Change in length of front piece = $\left[\frac{\text{Final front length} - \text{Initial front length}}{\text{Initial front length}}\right] \times 100 =$

% Change in length of rear piece = $\left[\frac{\text{Final rear length} - \text{Initial rear length}}{\text{Initial rear length}}\right] \times 100 =$

2. Describe what happened to planaria #2 over time, using your actual measurements.

3. Calculate the % change in length of your control planaria over time. *Express your answer as a percent.*

% Change in length = $\left[\frac{\text{Final length} - \text{Initial length}}{\text{Initial length}}\right] \times 100 =$

4. If your planaria (or sections of your planaria) died, please speculate as to why they died. What would you do differently next time?

5. How does this experiment account for multiple trials?

Conclusions – Team Project

1. Did your planaria that was cut in half (experimental planaria #1) regenerate? Refer to your % change in length figures in supporting your statement.

Was your hypothesis supported, refuted, or were your results inconclusive?

2. Did experimental planarian #2 regenerate? What happened?

Was your hypothesis supported, refuted, or were your results inconclusive?

3. Did the control planaria get smaller? Larger? Stay the same? Refer to the % change in length. What does this mean for your analysis of your other planaria?

Planaria Data Sheet – Class Results and Conclusions

1. Regeneration of experimental planaria #1 cut in half:

Group	% change in length Anterior	% change in length Posterior
Average		

2. Regeneration of experimental planaria cut in various ways #2

Group	Cut Made	Results

Conclusions – Group Results

What trends did you see? Was there a difference between anterior and posterior?

Refer specifically to the data, mentioning the averages as well as the range of numbers (highest/ lowest) and the number of planaria that were used total. What can you conclude about planaria that are cut in half?

What can you conclude from the results of the 'free choice cuts'? Again, refer to specific examples.

Overall, what conclusions can you draw from this investigation regarding the ability of planaria to regenerate?

How do you think planaria actually DO the regeneration? What might be happening to their cells? How might regeneration be possible?

Last Thoughts

A university lab studying planaria conducted the following research. Use what they learned to answer the questions:

Part I:

- Planaria were exposed to irradiation, which killed all the dividing cells in the organism.
- The irradiated planaria lost their capacity to regenerate any type of tissue when cut.
- The irradiated planaria survived for several weeks on the virtue of their already-differentiated, non-dividing cells.
- The planaria eventually died.

Part II:

- Neoblasts were isolated from wild-type animals and injected into the irradiated host planaria.
- The hosts regained their capacity to regenerate all types of tissues.
- The host planaria survived.

What special function does the neoblast have?

Would neoblasts be considered totipotent, pluripotent or multipotent? Why?

What would humans need for regeneration to occur? (hint: humans don't have neoblasts)

Are there limits to human regeneration? Explain.

Planaria serve as a MODEL organism for understanding human stem cells. How might our understanding of planaria regeneration be applied to help humans?

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