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Effects of Drugs on Pulsation Rate of *Lumbriculus variegatus* (Blackworms)

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Abstract: In this investigative lab, students observe blackworm pulsation rate in normal conditions and observe how pulsation rate is affected by drugs. This lab stresses the circulatory system, but can also be used for homeostasis, behavior, toxicology, and nervous system labs. Part I guides the student through blackworm handling procedures and initial observations of the blackworm's behavior and circulatory system. Part II is a student-led investigation in which the students design and run their own experiments to test drug effects on pulsation rate. The students write their investigations as an informal report and orally present their design, results, and conclusions.

Keywords: blackworms, *Lumbriculus variegatus*, pulsation rate, circulatory system, blood vessels, student designed investigations

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This major workshop paper is dedicated to and in memory of Dr. Charles Drewes.

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Introduction

Background

Blackworms (*Lumbriculus variegatus*) are excellent organisms for studying the circulatory system and the effects of drugs on this system for three main reasons: their skin is transparent making it easy to observe pulsation rates, drugs quickly diffuse through the skin of blackworms thus providing immediate effects, blackworms are easy to maintain in a laboratory. In blackworms, the dorsal blood vessel pumps oxygenated blood from the posterior to the anterior end by muscular contractions in each segment. At any time, several pulsation waves travel the length of the worm at a constant rate. Much like in humans, the pulsation rate is regulated by the nervous and endocrine systems. Since many drugs affect these systems (e.g. nicotine mimicking natural neurotransmitters), they can affect the rate of pulsation in bloodworms. In this investigative lab, students observe blackworm pulsation rate in normal conditions and observe how pulsation rate is affected by drugs.

In addition to the blackworm circulatory system, this lab stresses the following skills: scientific process/inquiry, collaborative group work, critical thinking, verbal and written, data collection and analysis, and working with live animals. Part I is designed to teach blackworm handling and viewing procedures and to guide the student through initial observations of the blackworm's behavior and circulatory system. Part II is a student-led investigation in which the students develop their own hypotheses and design and run their own experiments. The students write up their investigations as an informal report and orally present their design, results, and conclusion at a later date.

In its current context, this lab exercise is completed in a two hour, non-major's lab course. The lab course is an introduction to biology that is meant to supplement the lecture course material. When this lab exercise is performed, the students are learning about the human organ systems in lecture, including the circulatory system. Prior to this lab exercise, the students have learned about the scientific process and have designed a mini-investigation, including formulating an hypothesis, identifying a control, identifying independent and dependent variables, analyzing results, and drawing conclusions. Therefore, the lab content and process is not difficult for the students to understand; however, the handling of the blackworms and the counting of the pulsation rate can be tricky. Therefore, it is necessary that students are given sufficient practice with calculating pulsation rate before coming to lab (by visiting the website indicated in the student outline) and given some time at the beginning of lab to handle the worms (15-20 minutes). Other prerequisite knowledge and skills required for this lab include microscope usage and evaluating outside sources of information on the internet.

This lab can easily be modified and/or expanded to a three hour and/or advanced biology lab (see instructor notes). It can be adapted for lab exercises focused on homeostasis, toxicology, environmental biology, behavior, or physiology. For example, students could calculate the Q_{10} of blackworms' pulsation rate, test one or more physiological responses to external stimuli (pollution, acid rain, exercise, salinity, etc.), observe regeneration of blackworm fragments, or explore acute and chronic exposure to a toxicant.

There are many resources available for learning about blackworms and learning how to handle them and experiment with them. In addition to the background information and reference publications in the literature cited, you can also find a lot about blackworms on websites, especially Dr. C. Drewes website: <http://www.eeob.iastate.edu/faculty/DrewesC/htdocs>. Additionally, teachers attending the 1996 Woodrow Wilson National Leadership Program have developed many similar blackworm lab activities, which can be found on various websites.

Lab Exercise Objectives

1. Identify blackworms.
2. Explain and identify key features and functions of the blackworm's circulatory system.
3. Describe blood vessel pulsations of a blackworm.
4. Measure pulsation frequency and velocity.
5. Explain the effect of drugs on the circulatory system of blackworms.
6. Design and implement an investigation using blackworms.
7. Present results and conclusions both in writing and orally.

Timeline for Lab Activities

Culturing/buying worms	Start culturing 2-4 weeks in advance
Cutting worms	24-48 hours in advance
Making solutions	24 hours in advance
Time needed for preparing lab	~5 hours if viewing slides have been previously made

In-lab timing:

Introduction	10 minutes
Selecting and Handling worms	15 minutes
Determining Baseline Rate (Part I)	30 minutes
Designing Experiment (Part II)	20 minutes
Running Experiment	45 minutes

History of Blackworms in Biology Teaching Labs

Prior to 1996, *Lumbriculus variegatus* was well known among fish hobbyists. Thanks to Dr. Charlie Drewes, the wonderful world of blackworms was introduced to biology teachers all over the nation by Dr. Drewes' Carolina Tips article in 1996 and by his guest appearance (as an instructor) at the 1996 Woodrow Wilson Institute at Princeton. Teachers from this institute have developed and shared many blackworm related lab ideas, which has made this lab exercise possible. Additionally, much information about culturing, handling, and viewing blackworms was gained through Dr. Drewes' website and other compositions.

Student Outline

Introduction

Purpose for This Lab

This lab activity serves three purposes: to introduce you to the circulatory system of blackworms, to demonstrate the effects of drugs on the circulatory system of blackworms, and to provide additional experience in designing and performing your own lab investigation. By the end of this lab, you will be able to identify blackworms, explain and identify key features and functions of the blackworm's circulatory system, describe blood vessel pulsations of a blackworm, measure pulsation frequency and velocity, explain the effect of drugs on the circulatory system of blackworms, design your own investigation using blackworms, and present your results and conclusions both in writing and orally (due at the end of the semester).

Function of a Circulatory System

A circulatory system is needed by any animal that is too large and/or complex to obtain essential chemicals by the process of diffusion alone. Most importantly, a circulatory system quickly transports nutrients, oxygen, and other important chemicals to all body cells. Circulatory systems have three components: circulating fluid (blood or hemolymph), a heart or pulsating vessel which pumps the fluid, and vessels through which the fluid travels. There are two types of circulatory systems, closed and open. **Open** circulatory systems have vessels that are open at one end allowing hemolymph fluid to flow out among the cells. Most mollusks and arthropods have an open circulatory system. In a **closed** circulatory system, the fluid is called blood and this blood remains within the vessels as it rapidly circulates the body. Vertebrates and annelids have a closed circulatory system. The pumping of blood or hemolymph in a circulatory system is achieved by regular muscular contractions. The rates of these contractions can be regulated either by hormones or by neurotransmitters released by nerve cells.

Lumbriculus variegatus

Blackworm is the common name for *Lumbriculus variegatus*, a freshwater oligochaete worm in the **phylum Annelida** (earthworms and leeches are also in this phylum). Blackworms can be found naturally in stagnant water along edges of marshes and ponds where they feed on small living and decaying organisms. You can also find these worms at local tropical fish stores since they are great food for pet fish. Blackworms are small worms, ranging from 4-6cm in length (~150 body segments with head region containing 7-8 segments) in lab conditions, and up to 10cm in length in their natural habitats.

Blackworms have several complex organ systems including a **closed circulatory system**, which transports nutrients and oxygen; a complete digestive tract; and a nervous system, which includes a brain and a nerve cord. Using their nervous system, the blackworm can respond very quickly to shadows, touch, and vibrations by swimming, crawling, or performing a body reversal (rapidly coils and uncoils to turn itself around). These worms obtain oxygen through their skin on their tail; hence the reason they can often be found with their tails hanging out at the water surface. Unlike many other animals, sexual reproduction is rare in blackworms; instead, it commonly multiplies by **fragmentation and regeneration**. The worms will simply split into two or more sections, and each section will grow a new head and/or tail. You may notice that some blackworms are darkly pigmented at one end compared to the rest of the worm – the dark area is the original fragment (Drewes, 2003; Drewes 1996).

Pulsation Rate of Lumbriculus variegatus

Today you will be observing the pulsation rate of blackworms. The blackworm has a large **dorsal blood vessel** that is very easy to see using a microscope because the skin of the worm is transparent. This dorsal blood vessel pumps oxygenated blood from the tail (which is usually kept towards the surface of water) to the head of the worm by using rhythmic muscular contractions. The blood returns to the posterior end of the tail via the ventral blood vessel, which is not pulsatory and is connected to the dorsal blood vessel via small vessels in the first 1-18 body segments of the worm. In addition, to aid in the pumping of the blood, most body segments have a pair of lateral, pulsatory vessels that do not connect to the ventral blood vessel.

At any one time, you can see several pulsation waves along the length of the worm. Blood vessel pulsation rate in blackworms is partially controlled by neurotransmitters that are secreted by nerve cells (very similar to control of human heartbeats). The frequency (how many beats/waves per minute) and the velocity (distance traveled per minute) of the pulsations can easily be calculated by observing the pulse in the middle section of the worm (Lesiuk and Drewes, 1999). Because the rate of pulsation is easily seen and calculated and some chemicals can easily diffuse through the worm's thin skin, it is easy to test the effects of exposure to different chemicals on the cardiovascular system of the blackworms. This is what you will be doing for the second part of today's lab. During the first part of today's lab you will be performing baseline observations of the behavior and pulsation rate of blackworms.

Safety Precautions, Disposal, and other Notes

1. Dispose of glass waste in the glass boxes
2. Handle organisms with care
3. Handle microscopes with care.
4. Report broken equipment, slides, etc. to the TA.
5. Making slides and cutting worms can result in minor wounds. Please take the necessary precautions to avoid injury and report all cuts, however minor, to the TA.

Pre-Lab Assignment

1. Before lab begins, you will need to become familiar with blackworms and how to accurately measure the dorsal blood vessel pulsation rate for the worms. Below is the URL for a website (Drewes, 2001) that you should access before lab this week. This website provides a close up view of a blackworm body segment (these are segmented worms) and shows you how blood pulsation occurs in a worm. Read the directions and answer the questions for BOTH the posterior end of the worm and the mid-body section of the worm.

<http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/INT-ANIMA-LvDBV-mid.htm>

2. What types of chemical compounds affect the heart rate of humans? Perform an internet search to find the names of at least two chemical compounds that affect the heart rate of humans. In what way does the heart rate change when humans are exposed to these compounds (increase or decrease?) and how does that change occur? Please remember to cite the name of any websites, books, articles, etc. that you use.

Part One: Baseline Observations

In this part of the investigation, you will observe “normal” behavior and basal pulsation rate for living blackworms. Make careful observations, sketch what you see, and record relevant data. Make sure that both people get the chance to observe the worms’ pulsation rates using the microscope! At the end of this initial investigation, you will be combining class data.

Important Notes

- The basal pulsation rate is generally greater at the tail end of the worm because many pulsations starting at the tail end never make it all the way to the other end of the worm. Therefore, when you observe the pulsation rate of the dorsal blood vessel, make sure to observe a mid-body section of the worm and to always view the same segment throughout the entire investigation.
- Never use tap water with these worms! The chlorine in the tap water is toxic to the worms. Use spring water and/or aged tap water for all parts of this experiment.
- Never use forceps or sharp objects to touch the worms – they are very fragile!
- Several factors can affect the behavior and the viewing of the worms = temperature, age, health, direct light exposure, etc. Therefore, talk to your lab instructor if you have problems with a particular worm.

Procedure

1. Fill both of your specimen bowls with spring water to a depth of approximately 2cm.
2. You will now select 5-10 worms that are equal in size. Avoid picking any worms that have recently regenerated (worms that have a dark pigmented area and a lighter pigmented area). To remove your worm from the water, you will need to use a plastic pipette. Gently suck up the worm with a little bit of water and place into your specimen bowl.
3. You will also need to select 5-10 cut worms from the bowl at the TA desk. The anterior third and the posterior third of the worm were cut off yesterday and placed in another bowl for regeneration. You will be using the middle third of the worm for the second half of this lab since this is easier to work with than a whole worm. The worms were cut yesterday to give the ends time to heal for today’s lab. Before using these worm segments for the second part of today’s lab, you will need to determine if the pulsation rate of the middle third is similar to the pulsation rate of the mid-body segments of a whole worm. **Why do you think we need to determine this?**
4. Obtain 5-10 middle third worm segments and place them in the second specimen bowl.
5. Watch the whole worms – what are they doing? How are they moving? Are they clumped? Swimming? Record in your notes what you observe. Can you identify the head end of your worms? The head segments are generally darker, wider, and more blunt than the tail end. When you observe these worms with the microscope, you should also be able to tell the difference between the head and tail ends by how the blood vessel pulse moves (from tail to head).
6. Remove a whole worm from your bowl with a plastic pipette.
7. Place the worm into the trough on the well slide. Gently remove any excess water with a chemwipe and place a coverslip over the worm. Wait a minute or two for the worm to adjust to the trough (stop wiggling).

8. Place the slide on the microscope and observe the worm at scanning power (4x) or using a stereoscope.

*NOTE: Since intense light exposure can fry your worms and/or make them hyperactive, use a low amount of light and avoid exposing your worms for long periods of time to the light.

9. Find a segment as close to the middle of the worm as possible. Count the number of pulsations that pass through this point on the worm over 30 seconds. Multiply this by two to get rate per minute. Repeat this procedure two more times. Then, find the average pulsation rate per minute (record data in Table 1).
10. Place this worm into a weigh boat containing a small amount of spring water (just enough to cover the worm). Label the weigh boat so that you can recall which worm is where.
11. Obtain another worm and have your partner run through #6-9 with this worm. This worm should be placed in a different weigh boat. Record the data in Table 1.
12. Run through #6-9 using a third worm. Place this worm into its own weigh boat too. Record your data in Table 1.
13. Now, run through #6-9 using three of the middle third segment worms. Record your data in Table 1.

Table 1. Basal Pulsation Rate for Uncut and Cut Blackworms

Uncut Blackworms	Average Pulsation Rate
1	
2	
3	
Average Rate for Uncut:	
<hr/>	
Cut Blackworms	
1	
2	
3	
Average Rate for Cut:	

Discussion

1. Put your results on the board. Your TA will calculate the average pulsation rate for both the cut and uncut worms for the entire class. What is the class average for the cut worms? For the uncut worms?
2. How did the class average pulsation rate for the cut worms compare to the class average for the uncut worms?

3. Explain why the results are different or similar. What could have caused a difference if there is one?
4. For data to be reliable, your data need to be accurate and reproducible. How have you achieved this?

Part Two: Investigating the Effects of Drugs on Pulsation Rate in *Lumbriculus variegatus*

In this part of the lab, you will get the chance to design your own investigation. Certain chemicals and drugs can greatly affect organ system function. Today, we will be looking at the effect of drugs on the circulatory system of blackworms. Based on the research you performed for pre-lab and the chemical compounds that your TA has available, you will design an investigation to see how pulsation rate changes in response to exposing your worms to drugs. You have several options on how to design this – you can investigate the effects of one or more drugs, investigate the effect of different concentrations of a drug, investigate the effect of the length of exposure time to the drug, and/or you can investigate the length of time it takes for the worms to recover from the effects of the drug.

Before beginning your investigation, please review information about setting up an investigation, paying close attention to the necessary factors for a sound investigation (control, limiting variables, etc.). Also, before you begin your procedures, talk to your TA about hints and suggestions for running this type of investigation.

For your investigation, use the pre-cut worms (the middle body segments that you used in the baseline observations earlier). If you need to use more worm segments, remember to first obtain a baseline pulsation rate for each worm! Also, make sure to rinse your worms before placing them in the trough and/or rinse your trough between observations so that you do not contaminate other worms.

Design Your Experiment

Before you begin, describe your experiment below and show your description to your TA. Do not proceed with your experiment until your TA has given you the go-ahead.

- What would you like to investigate?

- What is your hypothesis?

- What is your dependent variable (what will you measure)?

- What is your independent variable?
 - Why do you think this independent variable will affect the pulsation rate?
 - How do you think this variable will affect the pulsation rate?

- What is your control? Be very specific!

- How will you include replications?

- What results would support your hypothesis?
- Describe your methods:
- What materials will you need?
- What do you predict will happen?

Perform Your Experiment

As you carry out your experiment you will want to record your procedures, results (including table/s to collect data and observations), and conclusions in a notebook. Be thorough and detailed as you record your results. If you have problems, questions, and/or errors during the experiment, be sure to write these down. Use the following information to guide you in writing your results and your conclusions:

- Results - Describe your results in general. Do not explain why you got these results yet. Decide how best to present your results – as a table and/or as a graph – and then complete your tables and/or graphs before you interpret your results. You can use excel to design graphs.
- Conclusions –
 - Look back at your hypothesis and look at your tables and graphs. Do your results support or refute your hypothesis? Explain by using your data as evidence.
 - Do your results match what you predicted above? Why or why not? Explain. If they are not what you predicted, explain what may have occurred.
 - If you had an opportunity to redo this experiment, how might you do it differently to make it more convincing?
 - Answer the summary questions below

Summary Questions

1. What was the reason for using more than one animal for each test? Did all animals respond in the same way? Why or why not? What factors might influence individual response? What implications does this have for the effects of drugs on humans?
2. Describe how the drug affected pulsation rate. Why do you think your results occurred?
3. Would your drug be classified as a depressant or a stimulant? Why?
4. What behavior characteristics did you note? Are they different than the behavior of the unexposed worms?

Cleanup

1. Return all worm segments exposed to chemicals to the recovery bowl (do not dump chemicals into this bowl –rinse your worms in spring water first).
2. Return all unexposed worm segments to the regeneration bowl.
3. Return all unexposed whole worms to the other bowl.
4. Dispose of chemical waste appropriately
5. Clean off all slides really well
6. Turn off your microscope, clean the lenses with microscope lens paper, and put away your microscopes

Poster Presentation Information

Refer to Table 2 for grading information. You should present the sections of the poster on one posterboard. You may use illustrations, pictures, drawings, etc. – be creative but don't include irrelevant information! Use a large font – something that can be read from 5 feet away. Single spacing is fine. Label each part well (and in bold). All written parts should be in complete sentences and in paragraphs (no bulleting).

Introduction:

The purpose of this section is to explain why you are performing this experiment and to provide background information necessary to understand the framework of the experiment. Here you want to describe the role of the cardiovascular system and discuss factors that can influence it (including “how” and “why” these factors may change the pulsation rate). You also want to describe the organism we are studying in lab and why we chose to use this organism. Then, you want to provide information about the chemical you chose to test. Explain what is known about the chemical and then state what you expected to see when you tested the chemical. Why did you expect this? The last paragraph typically states your original question.

Materials and Methods:

This section should describe in moderate detail how the experiment was performed, and should include the explanation of controls and the number of replicates performed.

Results:

This section is where the data is presented. Data should be presented as tables and graphs with titles/brief explanations. No conclusions should be in this section.

Conclusions:

The conclusion should explain the results that you obtained and if your hypothesis was or was not supported. This section should also include answers to any summary questions from the lab.

References:

Cite any outside information that you used when writing the introduction, material and methods, and/or conclusions. See examples in book for correct format.

Reflection Paper

Describe what you learned from this lab/process. Discuss what you liked about the lab and any ways that this lab might be improved. 1-2 pages double-spaced.

Table 2. Grading Rubric for Investigation, Poster Presentation, and Reflection Paper

	1 point	2 points	3 points	4 points	Score
Prelab Assignment	In complete in more ways than one	Partially incomplete, no effort shown, no references	Does not thoroughly discuss #2, does not have accurate answers for #1, and/or does not reference websites	Completed on time, correct information, #2 thoroughly discussed and referenced	
Question Investigated	Not related to topic and not testable	Addresses too many variables and/or not related	Not in correct format, but is testable and related	Directly related to prelab research findings, testable, correct format	
Experimental Design	Lacking 3 or more of the criteria for a good experiment	Lacking 2 of the criteria for a good experiment	Lacking one of the criteria for a good experiment	Includes control, only one experimental variable, design directly answers original question, other variables kept constant	
Poster					
Introduction	Question not identified and/or summary incomplete	Summary of background information is not complete	Identifies question. Summary of background information complete, but not clear and/or concise	Identifies question investigated. Provides a clear and concise summary of necessary info (see below)	
Materials and Methods	Not sequential, most steps are missing or confusing	Some of the steps are clear, most are lacking detail and are confusing	Most of the methods are understandable, some lack detail or are confusing	Clear and concise summary of methods used with adequate detail	
Results	Incomplete information including other problems	Mostly complete information, but inaccuracies, mislabeling, and confusion	Information accurate. Labels missing and/or information is not clear	Tables and graphs complete, accurate, well labeled, and clear. Clearly written summary of trends	
Conclusions	Presents an illogical explanation of findings and doesn't address original question	Presents an illogical explanation of findings	Presents an explanation of findings and addresses original question, but is not clear and/or complete	Presents a clear, complete, and logical explanation of findings, with evidence, and addresses the original question	
References	Missing citations and not in correct format	Missing citations but in correct format	Citations not in correct format	Everything outside source is cited, citations are in correct format	
Grammar	Very frequent grammar or spelling errors	More than 2 errors	Only one or two errors	All grammar and spelling are correct	
Organization	Disorganized, incorrect placement of parts, not neat	Somewhat organized, lacking flow, incorrect placement of parts	Mostly organized, some parts are out of place or do not flow well	Very well organized, everything in correct place, good transitions, neat	
Creativity	Lacking creativity	Creative, but the creativity causes design problems	Poster and question investigated somewhat creative	Poster is creative and question investigated is unique and/or innovative	
Reflection Paper	Incomplete, no depth, not interesting	Somewhat incomplete and lacking depth	Complete, but lacking depth and/or creativity	Complete, interesting, creative, well thought out	

Materials

Materials listed are for a class size of 20 students working in pairs. There are two student pairs at each lab bench.

Culturing Blackworms

- *Lumbriculus variegatus* (need approximately 10-20 worms per group). These can be ordered from Carolina Biological Supply (# CE-14-1720), Flinn Scientific, Inc (#LM1220), or can be purchased at a local aquarium store. See appendix B for culturing instructions.
 - “Starve” worms at least 2 weeks in advance of the lab
- Spring water or aged, dechlorinated water (let tap water sit in an open container for ~2 weeks)
 - Worms are very sensitive to chlorine
- Small aquaria or buckets, large finger bowls, or 2 liter pop bottles for holding worms
- Brown paper towels
- Sinking fish food pellets

Prep Materials

- Single edge razor blades (new)
- Disposable petri dishes, Ward’s Biology (#19-7100)
- Filter paper, 90mm diameter, Ward’s Biology (#15-2815)
- Dissecting microscope
- Finger bowls to separate worms
- Standard Plastic pipets, Ward’s Biology (#18-2971)

Labroom supplies (front or back bench)

- *Lumbriculus variegatus*, uncut worms in one bowl, cut worms in another bowl
- Nicotine, caffeine, alcohol, and/or other drug solutions (labeled) (see Appendix A for recipes)
- Labeled plastic pipets
- Large finger bowl labeled “Rehab” and one labeled as “Regeneration”
- Graduated cylinders (1-ml and 10-ml)
- Latex gloves
- Spring water or aged, dechlorinated water
- Computer with internet access

Supplies at lab bench

- Parafilm trough slides or Tape well slides, 2 per group. (See Appendix B)
- Coverslips – heavy transparency or plastic preferable
- Chemwipes
- Weigh boats, 10 per pair, Ward’s Biology (#18-1453)
- Several standard plastic pipets
- Compound microscope and/or stereoscope, 1 per group
- Petri plates to raise worms away from light of stereoscope
- Widgets, 1 per group (See Appendix B)
- Eye droppers for solutions
- 100-ml beakers for solutions
- Stop watch – one per pair
- Cotton swabs
- Microrulers (See Appendix B)

Notes for Instructors

Lab Design Information

The prelab assignment included in the student outline is a way for students to start making observations about the blackworm's circulatory system and about how drugs can affect heart rate. Before this assignment is given, however, the students will need instruction on how to evaluate outside sources of information on websites. They will also need to be reminded to cite all websites that they reference. Coming to the class with the observations and background research complete, and an idea of what type of drug they would like to test, saves time and prepares the students to make their hypotheses. After Part I, the students formulate these hypotheses based on their observations, research, and supplies available. The students will need guidance during this part so that their hypotheses are specific and testable.

Before Part I begins, which is performed and discussed as a class, you may need to review basic information about circulatory systems of annelids, "heart" rate, blackworms, designing a hypotheses and making predictions, writing up lab results and conclusions, safety issues (see above), and the purpose for the day's activities. It will be important for the instructor to do background research before teaching this lab to help guide students and work out issues that come up with using the worms. Refer to the literature cited section of this paper for some of the best references on blackworms.

Purpose for Part I:

- To have baseline data (rates before treatment) to compare with experimental data.
- To practice handling and observing blackworms. Students will observe both behavior of the worms and dorsal vessel pulsation.

Purpose for Part II:

- To design and run an investigation to test the effects of "x" on pulsation rate. Purpose is not to kill the worms, but to determine the effects of sublethal concentrations of a drug on dorsal vessel pulsation rate (and behavior).

Before having the students make their hypotheses for Part II, ask them what they found out about drugs and the effects they have on heart rate. How do drugs affect heart rate? Is it dependent on concentration and/or exposure time? How might exposure to several drugs change the effect? Can blackworms recover from exposure? How long does it take? Can blackworms die from exposure? Would acute exposure affect the pulse rate differently than chronic exposure? These are all good questions for the students to consider in making their hypotheses for their experiment (Part II).

You can have the students proceed with their hypotheses and experiments in one of several ways:

- You pick which drug they test and how they test it.
- You pick which drug they test, but they choose what they want to test (concentration, type of drug, exposure time, recovery time, etc.).
- Students pick which drug they test, but each group has to pick a different drug.
- Students pick which drug they test and it doesn't matter if they possibly end up all picking the same drug (you could have results about other drug effects available for them to see).
- Either of the last two option above, but you choose how they test it.

Possible Extension Activities:

- Calculating vessel diameter and pulsation velocity using microrulers (See Appendix B)
- Calculating Q_{10} of pulsation rate
 - $Q_{10} = \text{rate at Temperature}_1 + 10 \text{ degrees} / \text{rate at Temperature}_1$
- Chronic versus acute exposure to drugs
- Lethal and sublethal levels of drugs
 - Students can determine the concentration of a particular drug that would evoke the following responses:
 - Low concentration = little to no response in pulsation rate
 - Medium concentration = near maximum response
 - High concentration = no increase response, but still sublethal

Viewing Blackworm pulsations

Look for worms that are healthy (wriggling), not recently regenerated (colorful), and “starved” (guts are not dark). Use a plastic pipet to transfer the worms to their viewing slides (either the parafilm troughs or the tape wells). Immediately after placing the worms on the slides, suck up extra water with a fine tipped pipet. The water level should be even with the top of the well. Use tissue paper to soak up extra water around the edges of the well. If the worm is not quite in the well or is wriggling out, encourage it back in with the widge. Wait a minute or two for the worm to settle down. Then, place the slide on the microscope and view using scanning power. Use a minimal amount of light so the worm does not get overheated. Discern which way (to the left or to the right) that the pulse is moving so that you can determine which end is the posterior end (remember, blood flows from posterior to anterior). Pulsation rates may be higher at the posterior end of the worm because some of the dorsal vessel contractions die out before reaching the anterior end of the worm. Therefore, it is important to monitor the pulsation rates at the same location for each worm and for each time on the same worm.

Typical Data for Cut Vs. Uncut

Each number is an average for pulses/minute of three worms counted by each student group:

- Cut = 22, 13, 12, 16, 21 = 17 pulses/minute
- Uncut = 17, 13, 13, 13, 27 = 17 pulses/minute
- Cut = 18, 18.7, 14.6, 12, 17, 15 = 15.9 pulses/minute
- Uncut = 21.7, 10.6, 16, 14, 13.8 = 15.2 pulses/minute

How drugs affect the pulsation rate of Blackworms

Different chemicals (drugs) can have different effects on the pulsation rate of the dorsal blood vessel. Their effect can occur by mimicking natural neurotransmitters that bind to “heart” receptors, changing the propagation of action potentials, changing the amount of neurotransmitters released, changing the amount and type of hormones that are released, blocking ion channels, or by directly affecting muscular contractions. Most drugs enter the bloodstream of blackworms by diffusion through the skin. Thus, they also wash out of the bloodstream once placed back in a dilute environment.

- Just like many other drugs taken recreationally, nicotine mimics a neurotransmitter that controls pulsation rate. Nicotine is an acetylcholine agonist, so it increases the pulsation rate of the dorsal blood vessel (depending on the concentration of nicotine).

- In humans, caffeine inhibits the enzyme phosphodiesterase, thus allowing cAMP levels to go up and ultimately increasing the heart rate. In blackworms, it is not clear if the increase in pulsation rate due to caffeine is a direct or indirect effect.
- Alcohol acts as an ion channel blocker, thus decreasing the pulsation rate of the dorsal vessel.

Blackworm responses to solutions

- Response to caffeine – At low concentrations, worms may clump. As concentration increases, worms become very active. They may curl up and stretch out at higher concentrations. Pulsation rate will increase. Most worms recover within 15 minutes and all recover within one day.
- Response to alcohol – Worms will become inactive as concentration increases and will be less likely to clump. Worms may straighten out, with their ends curled, in higher concentrations. They will not be able to swim as well. Their pulsation rate will decrease. Worms at the lower concentrations will begin to recover within 15 minutes. Most all worms will recover within one day.
- Response to nicotine – Worms become more active, but do not clump. At moderate doses, the worms may be less active and twitch. In high doses (0.1 mM), paralysis (worm will be stretched out and motionless) may occur. The pulsation rate may not show an increase at the lowest concentration, but will increase at the middle and high concentrations. Most worms begin to recover within 15 minutes and all recover within one day.

Tips for Instructors

- Suggestion for beginning the lab (to engage)-
 - Have the students locate their pulse and ask them what they are sensing. Then, ask them how they could change the rate of their pulse. Once they start bringing up many types of drugs, ask them how drugs might affect the rate.
- When students first obtain their worms, have them place them in a weigh boat with just spring water. Tell them to take some time making initial observations – which end is the tail end? How do you know? What is the behavior of the worm? Does the worm swim? How? How is the worm responding to its new environment? Etc. Lead a class discussion about their observations.
- To get the worm to stay in the well and to coax it into a good position for viewing, gently use the widget or a piece of hair.
- Have one person view worm and count pulses while the other keeps track of time. Switch roles often and make sure both students are relatively consistent when completing the baseline rate count.
- Students can mix their own dilutions of the stock solution, or you can have dilutions already made for them. They could also try other dilutions than just the ones recommended in the recipes in Appendix A.
- Tell students to be careful not to transfer liquid from one container to the next as they move the worms. Students also need to discern between pipets to reduce contamination.
- Wells need to be rinsed thoroughly with distilled water between worms

- Encourage students to thoroughly think about their control for their experiment. Moving worms to and from dishes and slides could affect them; thus, it may be necessary to do the same for control worms.
- You may want to check students' procedures they have written down before letting them proceed with their experiment. They may need a little guidance. Avoid telling them what to do; instead, ask leading questions to help them develop a more sound experiment. Or, let them make mistakes and talk about sources of errors and mistakes at the end of lab.
- If comparing pulsation rate of cut to uncut worms in Part I, have students put data on board.
- If students end up experimenting with more than just the few specimens they used for finding the baseline rate in Part I, you will need to encourage them to find the baseline rate for all of the other worms that they are going to use.
- Students may think that a very small change in pulsation rate is meaningless. Remind them that even very small changes in an organisms' body can have significant consequences. For example, slight changes in body temperature, calcium levels, blood pressure, etc.
- Potential pitfall – Students will not notice a change or will have a myriad of problems resulting in poor results. For these reasons, have the students also note changes in behavior so that, worse comes to worse, they can write about behavior changes in their lab reports.
- If using statistics to analyze results, students will need to use at least 5 worms per treatment. Students can use a paired difference t-test to compare before and after exposure.
- To save time, be strict about allotted times. Also, have data for the control group (worms that are never treated but are transferred back and forth) already available.

After Lab Notes

- Rinse off worms well. Cut worms that were in treatments go into "Rehab" bowl (do not dump treatment chemicals into this bowl!). Untreated cut worms can go into "Regeneration" bowl.
- Rinse off and dry viewing slides, weigh boats, and other containers.
- Throw away plastic pipets.
- Wipe microscope lenses with lens paper and turn off microscopes.

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About the Author

Kelly Bohrer received a B.S. in Environmental Biology and an M.S. in Biology from The University of Dayton, where she is currently the Biology Lab Coordinator. As such, she coordinates the activities of 4 lab courses per semester; teaches biology labs, introductory courses, and a graduate course on pedagogy for teaching assistants (TA's); supervises TA's and prep assistants; and develops innovative lab curricula. Her research interests include wetland ecology and laboratory pedagogy. She has recently received several grants to enhance laboratory experiences for non-majors and pre-service teachers and to develop a university wide graduate teaching assistant orientation.

Appendix A: Recipes for Drug Solutions

Make all solutions with spring water or aged, dechlorinated water. Use all solutions within 24 hours. Solutions can be stored at room temperature. The dilutions are designed to give a small effect at the “low” concentration and a more pronounced effect at the “medium” solutions. The “high” solutions should show that either the threshold concentration has been reached or that the exposure response has reached maximum (but still sublethal). Actual responses will vary depending on other factors.

Caffeine Stock Solution

- Use Vivarin tablets, NOT NoDoz!
- 200-mg caffeine/tablet
- Make a 5mM stock solution by crushing 2 caffeine tablets and add 412-ml of spring water (or aged water). Dissolve tablets with stirring and heating if necessary.
- Use appropriate amounts of the stock solution to make 500-ml quantities of 0.1 (low), 1 (medium), and 5 mM (high) solutions.

Nicotine Stock Solution

- Cigarettes - regular length and strength, NOT menthol, 100's, or ultralights
- 1.1mg nicotine/cigarette
- Make a 0.1mM stock solution by stirring the tobacco from 10 cigarettes in 680-ml of very warm spring water for 20 minutes. Filter the solution. You will lose about 50-ml of the solution when filtering.
- Use appropriate amounts of the stock solution to make 500-ml quantities of 0.01 (low), 0.05 (medium), and 0.1 mM (high) solutions.

Alcohol Stock Solution

- Vodka = 40% alcohol
- 1 mM alcohol = 2.6%
- Mix 32.5-ml of vodka and 467.5-ml of spring water to make the stock solution.
- Use appropriate amounts of the stock solution to make 500-ml quantities of 0.1 (low), 0.5 (medium), and 1 mM (high) solutions.

Other Possible Drug/Toxicant Solutions

- Diet pills, cold medicine, Tylenol, acetylcholine, epinephrine, lidocaine, glucose, sugar substitutes, saline solution, detergents, pesticides, etc.
- Crush and dissolve tablets in spring water. Make a high, medium, and low solution. Groups could work to find the concentrations that lead to little response and maximum response.

Appendix B: Preparation Notes

Culturing Blackworms

Fill a bucket, small aquarium, or large finger bowl with 2-3 inches of aged, dechlorinated water (or spring water). Add healthy worms (about 100) to the water and then layer the water with several small pieces of brown paper towel. Every week add one to two (depending on size of aquarium) pellets of sinking fish food. Do not overfeed! As water evaporates, add spring water to the original level. When the water begins to appear cloudy and/or starts to stink, slowly pour off as much of the water as possible without losing the paper towel pieces or the worms. Rinse the worms and paper towel once (with aged water) and then refill the aquarium (to 2-3 inches) with fresh water and a few new pieces of brown paper towel. If you are not using the worms for a while, split the culture or feed some of the extra worms to fish (the culture should double every 2-3 weeks and more quickly with slight agitation). This culture should live for a long time following these procedures.

Handling Blackworms

Worms are best handled by sucking them up with a plastic disposable pipet. Blow out the air in a pipet, place the pipet at a 45 degree angle, lower it to the bottom, and quickly suck up 1 or 2 worms at their head ends. If you dismember any of the worms in the process, just leave the pieces in the culture to regenerate.

Using Cut Blackworms

Blackworms that have been fragmented tend to move around less. Typically, the pulsation rate of a newly fragmented worm is close to the pulsation rate of a whole worm. At UD, we have the students actually verify this before “choosing” to use cut worms for their experiment. Each student group measures the pulsation rate of three cut and three whole worms (in approximately the same region of the worms), and then pool their data with the rest of the class. Then, as a class, we can decide if the pulsation rates are close or not. To save time, it may be best to either tell them that this is so or to have data available for whole worms and have the students see for themselves. If you do choose to use cut worms, these worms should be cut at least one day in advance so that the ends are healed. Select whole worms that are healthy and full-sized and cut them into thirds by placing them on a piece of saturated filter paper in a petri dish and cutting them with a clean, sharp razor blade. Keep the middle segments for the experiments and put the other two segments back into your culture so they can regenerate. Keep the middle segment worms separate from the whole worms and place both into separate bowls with fresh spring water for the lab exercise (label the bowls as “cut” and “whole”).

Making “Widgets” (for moving worms)

The following directions for making widgets is adapted from “A Toolbox for Working With Living Invertebrates,” by Dr. Charlie Drewes. This article can be found in ABLE’s 2004 proceedings.

1. Materials: applicator stick (handle of a probe works well), rubber band, scissors, and tape.
2. Cut, at an angle, a piece of rubber band that is one inch long.
3. Attach the rubber band to one end of the applicator stick with tape, leaving _ inch of rubber band beyond the end of the stick.

Making Viewing Slides

The procedure for making tape well slides is also presented in the article listed above. You can also find directions, and pictures, for both widgets and slides at

<http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/> (scroll down to “Gadgets & Technical Information”)

Tape Well Slides:

1. Materials: clear plastic tape (Scotch Colored Plastic Tape, Clear, 0.75” X 125”); forceps; single edge razor blade; heavy scissors; heavy-duty, flexible clear plastic (or glass microscope slides); pen; ruler
2. Using a pen and a ruler, mark off desired size slides on the plastic sheet.

3. Place a long strip of tape over the plastic sheet, ensuring that there are no bubbles
4. Add multiple layers of tape (4-5).
5. Using a ruler and razor blade, make vertical cuts to define the well sizes for holding the blackworms (3-mm deep and wide and 4-cm long)
6. Using a forcep, carefully lift the tape layers covering the desired well.
7. Cut out the “slides” from the plastic sheet.
8. On another piece of clear plastic, mark off and cut out rectangles to act as cover slips for your slides (make them a little smaller than your slides).

Parafilm Trough Slides:

1. Materials: single edge razor blade, parafilm, glass microscope slides, ruler, metal surfaced hot plate, glass plate (~same size as hot plate), forceps, glove for hot items
2. Put hot plate on low.
3. Lay out glass slides, side by side, on glass plate to about 6” long.
4. Cut out pieces of parafilm that are 4” X 6”.
5. Place several layers of parafilm (6-8) on the glass slides and press down on the parafilm to make sure the sheets stick to each other and the glass slides.
6. Put glass plate on hot plate and let it warm up for ~5-10 minutes. During this time, use parafilm backing to press the softened parafilm against the slides. Try to remove all air bubbles and make sure everything is sticking together.
7. Once the parafilm just begins to get clear and soft, remove the glass sheet (with gloves) and *carefully* place it on the counter.
8. Using a ruler and razor, make cuts in parafilm to define the well sizes for holding the blackworms (3-mm deep and wide and 4-cm long). Make long wells for whole worms and short wells for cut worms. You can put two short wells and one long well on each slide.
9. Use forceps to remove the parafilm from the cut wells.
10. If part of the parafilm lifts during this time, simply reheat the slide on the hot plate and press down on the parafilm.

Making Microrulers

- Visit www.eeob.iastate.edu/faculty/DrewesC/htdocs/microruler-links.htm

Safety Issues

- Clearly label the contents and concentrations of all chemical solutions, including stock solution and dilutions (remind students to do this).
- Read the Material Safety and Data Sheets (MSDS) for chemicals being used. For chemicals that are health hazards, including nicotine, wear gloves and minimize contact.
- Properly dispose of all solutions and all materials exposed to solutions.
- Scrub and clean all glassware and wipe down all benches with ethanol at the end of lab.
- If accidentally cut, wash the area thoroughly.
- Handle microscopes appropriately. Use lens paper to clean lenses before and after use.