Cardiovascular Inquiry Using *Lumbriculus variegatus,* Mudworms

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Editor's notes:
The APS encourages teachers to give students a copy of the "ABC" (Appropriate, Beneficial, Caring) rules for use of animals in the classroom, to discuss the rules, and to ask students to sign the "ABC" rules contract (see References). Also, teachers should have a plan for short term care of the animals (with supporting references for appropriate care guidelines) and for disposal or long-term care of all classroom organisms.

Teachers should carefully review any stimulus or environmental change for an animal being used in experiments or observations before students are allowed to use that stimulus. This is especially important if the stimulus could cause pain or distress to the organism. Teachers may be able to identify a less stressful stimulus for the students to use in their experiment.

Purpose:
Students in grades 9-12 can use this activity to develop a controlled experiment and to examine the effects of an environmental factor on the pulse rate (heart rate) of a mudworm.

Objectives:
Students will be able to:

- design a properly controlled experiment.
- present the results of the experiment in a group oral report and as an individual lab report written according to class standards.
- describe basic anatomy and behaviors of a mudworm.
- describe the effects of stimulants and depressants on the pulse rate of a mudworm.
- explain how environmental factors affect pulse rate.

Materials: (for a group of four; could be done in pairs if one has the equipment)

- Minimum of 12 mudworms. These can be ordered from Carolina Biological Supply, (800) 334-5551.. An informational sheet will be sent with the order. These worms can sometimes be obtained from local pet stores. They also are known as California Black worms and are used as fish food. Students should use worms that are more uniform in color. Worms that have undergone recent regeneration have easily seen light end(s). These worms should not be used.
- binocular scope or good hand lens.
- four (4) or more petri dishes (non-sterile) or other similar shallow container.
- plastic pipette with the narrow tip cut off to make a larger bore opening.
- a probe: round applicator stick with a 1-inch piece of rubber band attached to one end.
- 6 cm x 6 cm paraffin block.
- 100 ml of concentrated stimulant or depressant solution.
- distilled or spring water, graduate cylinders and flasks for making dilutions.

Data Collection:
Because of the potential for worms to be active even when in the paraffin chamber, students can count for 15 seconds instead of for 30 or 60 seconds. If students find the pulse rate difficult to obtain or if the availability of binocular scopes is limited, there are other data that could be collected. Students do not need to limit themselves to just one set of these observational data.

> **Swimming Behavior:** Normal swimming is in a corkscrew fashion using both a clockwise and a counter-clockwise movement. Students could use their probe and gently touch either the posterior or anterior end and write down their observations, noting direction of movement, duration, coordination, etc.
>
> **Crawling Behavior:** On a moist towel or a piece of filter paper the worms will use peristaltic crawling to move. Again, the worm can be gently probed and reaction can be recorded.
>
> **Body Posture:** Are the worms clumped together? Are their bodies all stretched out straight? Do the worms alternate between being stretched and coiled together? Is one end or are both ends curled?

General Activity: Are the worms actively moving about? Are some moving faster than others? Is their response to a touch the same? Do some need more probing to get a response than others? Is the anterior or posterior end more sensitive to probing?

Preparation and Procedures:

Mudworms: If you order them from Carolina Biological Company, specific directions for the care of these worms will be sent. In general, one only needs a small aquarium with 5-8 cm of spring or distilled water; sometimes aged water can be used. Put strips of a brown paper towel on the bottom of the tank and use an aquarium air stone. Feed with 1-2 sinking fish food pellets but do not over feed. (I kept mine in the refrigerator over the summer and they survived.)

Editors Note: Be sure to have a plan, both for short-term care of these organisms and for either proper disposal or long-term care of the organisms.

Probe: Take a round applicator stick and tape half of the rubber band to one end. Use a sticky tape like masking tape or electrical tape.

6cm X 6cm paraffin block: Buy the paraffin blocks that are used in canning. Cut one in half and you will have two that will be the size needed. With a small needle or paperclip make a groove 4-5 cm long and about 1 mm deep. Some people embed a piece of dark thread into the groove.

Possible Solutions: The following are several solutions that can be used. The teacher can make up a stock solution of the highest concentration or can let students do the work. Maximum concentrations of nicotine, caffeine, and alcohol are based on Lana Hays' NABT presentation. If you make up concentrations greater than these, the worms will die, which is not the point of the activity. Before you give these solutions to the students, you can do a trial run with one worm to make sure you have not made up a lethal dose. If the worms stop moving it does not mean that they have died. Check their pulse or put them into clean water and look for movement. Movement typically starts within 15 minutes but for some concentrations it might take 24 hours.

Safety Note: The directions that are given are based on individual groups making up their most concentrated solution. If the handling of these drugs by students is a concern, then the teacher should check with the administration or science supervisor for permission. If it is decided that the students should not handle the raw drug, then the teacher will need to make adjustments to the protocol based on class size.

Gloves and goggles should be worn when handling the raw drugs. When all solutions are made remember to use distilled or spring water.

Nicotine

The amount of nicotine in a cigarette can vary. If you call the manufacturer they will give you this information. You do not want more than 1.1 mg per 100 ml of water. This is typically the amount of nicotine in one cigarette that is of regular length and strength. Put

the tobacco from one cigarette in 100 ml of warm water for 15 minutes. Stir occasionally or put it on a mechanical stirrer. This will make a 0.011 mg/ml solution.

Note: Activity levels and pulse rate tend to increase at a lower concentration but as one reaches the 0.011 mg/ml concentration paralysis and lack of pulse rate can occur.

Caffeine

Use an over-the-counter medicine for preventing sleep. Many of these tablets have 200 mg. Try to use a tablet that does not have additional flavorings. Crush one tablet and put this in 200 ml of water. You will have to heat the solution to get most of this dissolved. This will make a 1 mg/ml solution.

Note: Normally one sees an increase in pulse rate but sometimes because of the increase in worm activity, this can make counting the pulse rate more difficult.

Alcohol

Denatured alcohols have been treated to be toxic. If you want to use alcohol this then you must substitute a clear drinking alcohol, like gin or vodka and label it as alcohol. **Make sure that your administration will allow you to use this in your school.** You would add 25 ml of gin or vodka to 75 ml of water. This makes a 10% alcohol solution.

Note: Alcohol not only causes a reduction in activity and less response to stimuli, but also a reduction in pulse rate.

Antihistamine

Use 75 mg of Diphenhydramine HCl or similar antihistamine in 100 ml of water. Make sure you are using a medicine that has only the antihistamine in it. If you use the capsule, you can open the capsule and pour the drug into the liquid. Heating helps it to dissolve.

Note: Activity level can decrease with the antihistamine since it is a central nervous system depressant. However, my students have usually found an increase in pulse rate. This could be because antihistamines bind with H1 receptors, which cause smooth muscle contraction.

Decongestant

Use 120 mg of Pseudoephedrine HCl or similar decongestant in 100 ml of water. Make sure you use a medicine that has only the decongestant in it. Crush the tablets and heat the solution. Do not worry if all of the coating does not dissolve. If you desire, you can filter out these particles or just let them sit at the bottom of the container.

Note: Decongestants typically are stimulants that cause an increase in activity and pulse rate as long as the decongestant does not cause drowsiness.

Time Frame: two-four days

Day 1: Students need to spend some time discovering mudworms, including: complete adult vs. regenerating, intact vs. fragmented, anterior end vs. posterior end, dorsal blood vessel, behavioral actions, how to handle worms without fragmenting them and counting pulse rate. After this period of discovery, they should develop a protocol for testing a possible factor that could be measured by counting the pulse rate. The teacher should check the protocol before students are allowed to carry out their experiment. For

homework, students would write up the first part of their lab report: Problem, Procedure, Hypothesis, Data Chart.

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Day 2: Students perform the experiment, collect data and begin to analyze the validity of the data. Does the data provide them with a clear insight into what is happening? Is more data needed? Is another experiment needed to help answer the problem? If another protocol is needed, students should design the experiment and then check with the teacher. For those students who believe that the data has answered their problem, they could be asked to decide what could be their next question and design an experiment. Students need to see that answers to a problem are usually not answered with just a single experiment. Once a scientist has one answer it leads the scientist to another question. Science is a continual process unlike the cookbook lessons that we so often do in our school labs. The teacher will have to decide if another day will be allowed for running the experiment. Even if you do not have enough time to devote to a rerun of the experiment, I suggest that you at least allow the students the opportunity to go through the mental process of analyzing and redesigning their experiment.

Day 3: Students continue to collect data, if time is allowed. They should begin to prepare for their oral presentation and work on their lab report. If the teacher is unable to allow time for a second day of experimentation, then students would begin the report preparation on day 1.

Day 4: Students turn in individual lab reports or a group report if you use this method. Each lab group will give an oral report about their lab. As a class, critique what all of the experiments say in terms of pulse rate activity of mudworms. The teacher will need to decide the depth in which you want to discuss the physiology of the stimulants and depressants on the cardiovascular system.

Besides the basic physiology, one could discuss **dose-response curves** if students are familiar with logs. A dose-response curve plots the response on the Y-axis and the log of the dose on the X-axis. When this happens a sigmoid curve is produced. From this curve one can see what is the minimum and maximum amount of a drug needed to produce the effect. From the dose-response curve one can determine that concentrations of the drug below the minimum will have no effect, and concentrations above the maximum is a waste of the drug since the maximum effect has been reached and, oftentimes, these concentrations can be toxic to the animal.

One could also discuss the difference between **efficacy** and **potency**. It would be beneficial for the students to know these two terms since they are used in advertising. When two drugs produce the same maximal effect, they are said to have equal efficacy. If one drug's maximal effect is less than another drug, then the first drug is less efficacious. Potency deals with the amount of drug needed to produce a similar effect. If it takes a

smaller concentration of one drug to produce the same effect as another drug, then the first drug is more potent. When you compare these two terms, efficacy deals with the effect, the measured response (y-axis) and potency is an expression of the concentration of the drug (x-axis).

Another term that students hear is **tolerance**. Tolerance refers to the lessening of a response to a drug with repeated administration. So if one wants to maintain the same response, then one must administer more of the drug.

Safety:
Wash hands before and after lab. The major concern in using these drugs is that students should not put anything into their mouths. They need to understand the seriousness of using a drug in general and also in the classroom for an experiment. The use of goggles and gloves, especially when handling the raw drug, should be required. The students also need to be aware that just because they are using an invertebrate does not mean that we treat these animals any differently than we would our vertebrate pets. They need to be taught the importance of treating all living animals properly.

Questions to Ask:
1. Have students consider if the effects that they saw with their factor would be the same if they used a different brand of cigarette, antihistamine, etc.
2. What would be their control if they wanted to compare brands of a drug?
3. How would they design an experiment if they want to compare cigarettes, cigars, and chewing tobacco?
4. Even if you do not want to go into any depth about dose response curves, you could draw different curves that display efficacy versus potency. You could then have students explain what the difference is and, which gives them more information about a drug for the consumer. Which term is more relevant for the consumer -- efficacy or potency?
5. The test you performed was an acute test. Have the students discuss whether they think the results of their lab would be the same if they performed a chronic test. If they did a chronic test, which of their concentrations might they use? Which of their concentrations would they not use?
6. The test the students performed were on intact worms. Do you think that the results would be the same on a regenerating worm? What metabolic differences might there be between a regenerating worm and an intact worm? Would these differences cause the regenerating worm to react differently to the drug?
7. If you did not watch what happened to the worms after you finished the experiment and put them back into their normal environment, let them hypothesize what happens to the worms as they undergo detoxification? Does the concentration of the drug effect the rate of detoxification?
8. Because these drugs acted in this manner on a mudworm, do you think that the effects would be similar on other animals? What about humans?
9. Why do researchers do their experiments on other animals first before seeing the effects on humans?

Where to Go From Here:
This activity could be used during your human body unit or during your animal taxonomy unit. In your human body unit this activity would easily fit into your discussion of the cardiovascular system or drugs and their effects. When you do your animal taxonomy, if you would like to do something other than dissect an earthworm, this unit is appropriate. Not only would the students see some external and internal structures of the mudworm, but they would also learn some of the behaviors of these worms.

If you have the time to spend four or more days on this, you could have a wonderful opportunity for the students to experience the life of real scientific experimentation. By giving the students

the opportunity to continue to improve on their experimental design or to think through what would be the next logical question to ask, the students should begin to develop a sense of what a scientist does.

Suggestions for Assessment:

- Protocol check by teacher
- Check of Day 1 Assignment
- Group and oral reports
- Individual lab reports
- Questions on test
- Student self-check of participation within the activity
- Cooperative group assessment by students.

References and Resources:

1. Lana Hayes: NABT Presentation at the NABT National Convention, October 11, 1997-"Toxicants and California Blackworms"
2. Charlie Drewes: "Those Wonderful Worms," Carolina Tips, August 1996
3. Carolina Biological Supply: Mudworm Care pamphlet. www.carolina.com
4. American Physiological Society (APS). "ABC" rules for use of animals in the classroom. [Online]. Bethesda, MD: American Physiological Society, 2007. http://www.the-aps.org/education/K-12misc/animaluse.htm.

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Student Lab Sheet

Answer questions on a separate sheet of paper.

Day 1

1. Using a pipette, obtain a mudworm from the culture and place into a petri dish with a small amount (about 1 cm) of the cultured water. **Do not pick a worm that is dark and has lighter sections at one end or both ends.** This indicates a worm that has recently fragmented and is regenerating its missing parts.
2. The anterior end usually moves before the posterior end. Using the rubber end of the probe, touch your worm and determine which is its anterior end. Describe physical differences of the anterior end versus the posterior end.
3. Observe the swimming behavior of the worm.
    a. Touch the worm on the posterior end and describe what happens. You may have to repeat this several times so as to detect all aspects of their swimming.
    b. Probe the worm gently on its anterior end and describe what happens.
    c. Put your worm in a petri dish with moistened paper. Make sure all excess water is removed. Investigate its crawling by performing the following; you may have to repeat this several times so as to detect all aspects of their crawling.
        a. Touch the worm gently on the posterior end and describe what happens.
        b. Touch the worm gently on the anterior end and describe what happens.
4. Using the paraffin block, use the pipette to carefully place the worm on top of the trough. The excess water needs to be drawn back up in the pipette. Once the water is removed, the worm will sink into the trough or you can coax the worm in with the aid of the probe. Place your paraffin block under the dissecting scope and count its pulse rate in its dorsal blood vessel. Since there can be a variation between the two ends of the worm you might want to take your counts from the middle. Count pulse rate for 30 seconds and multiply by 2 in order to get beats per minute (bpm). If the worm is too active, you can count for 15 seconds and multiply by 4. Take several readings and record the average pulse rate.

Background Information:
Your lab group will design an experiment to test the effect of different concentrations of a cardiovascular depressant or stimulant, or another aspect of the mudworm's environment on its behavior and/or pulse rate. The data that you can observe are the following:

- clumping behavior: do the worms clump in a ball?
- swimming behavior: do they swim forward and backward in a normal fashion?
- crawling behavior: when probed, do they crawl forward and backward normally?
- pulse rate: how many beats/minute (bpm) occur?

As you observe these behaviors make note of activity level (faster or slower than the control worm) and individual position in the water such as stretched out, curled in a ball, ends curled, as well as anything else that might be considered an unusual response.

The chemicals available for use are alcohol, caffeine, nicotine, antihistamine, and decongestant. The stock solutions in class are considered to be 100% effective.

In designing your experiment you can use three worms in each of your variables and you should limit the number of variables to three or four, including your control group. Expose the worms to each variable for between 5 to 15 minutes. Make sure that you keep the time for all worms constant. During this time you can make observations of behavior. At the end of this period of time take their pulse rate if you are using this measurement.

After the exposure period has ended, place the worms in the recovery tank. After sucking up a worm, try to expel as much of the liquid as possible out of the pipette before squirting the worm into the recovery tank. This limits the amount of chemical added to the recovery tank.

Lab Protocol:
Your group now needs to decide your **problem**, your **independent variable**, **dependent variable,** and **constant variables**. Design your procedure, state your hypothesis, and construct data chart(s). Show the protocol to the teacher for approval. Make sure to write this all up for the next lab day.

Day 2

1. Conduct your experiment. Analyze your results.
2. Does your data give you an answer to your problem?
    a. If the answer is, “yes,” what would be the next question you would ask to give you a better understanding of how toxicants affect mudworms? Design an experiment to answer your new question.
    b. If your data does not answer your problem, what does this mean to you? Was there an error in your experimental design or did you make an error in following your procedure? What should you do next to give you better results? Design this protocol.
3. Graph the data from your lab(s) and discuss these results. Explain the role of your drug on the cardiovascular system and/or behavior of the mudworm.