A New Approach in Examining the Influence of Drugs on Pulsation Rates in Blackworms (*Lumbriculus variegatus*).

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Abstract: This investigative laboratory activity engages students in observing, recording, graphing and analyzing pulsation rates in a commonly used laboratory organism, blackworms. This activity stresses how various drugs can impact the pulsation rate in blackworms at varying concentrations. In addition, we have incorporated two new ways to view the blackworms under the microscope.

Key words: Blackworms, pulsation rate, *Lumbriculus variegatus*, blood vessels, capillary tubes

INTRODUCTION

Lumbriculus variegatus, or blackworms, are freshwater oligochaetes in phylum Annelida. They are an excellent organism for studying the regeneration of body parts, regulation of reflex activities, bioaccumulation and toxicity of environmental pollutants, and regulation of blood vessel pulsations (Drewes & Fourtner, 1990; Veltz et al., 1996; Bohrer, 2006; Fillafer & Schneider, 2013).

Like other annelids, blackworms have a closed circulatory system (Fig. 1). Blackworm blood is red, due to a hemoglobin-like pigment called erythrocruorin dissolved in the blood plasma (Jamieson, 1981). Two major blood vessels, one dorsal and one ventral, extend the length of the blackworm. Pulsations along the dorsal blood vessel (DBV) propel blood through the circulatory system. Because the body wall of the blackworm is transparent, it is possible to visualize the pulsation of the DBV using light microscopy (Lesiuk & Drewes, 1999). As in humans, the pulsation rate is controlled by the nervous and endocrine systems. Many drugs affect these systems and can have an immediate impact on the pulsation rate, based in part on how quickly they can diffuse through the blackworm's skin. Due to their simple body plan and ease with which they can be treated with compounds and the subsequent pulsation rate measured, black worms are an excellent model organism for the lab activity described below.

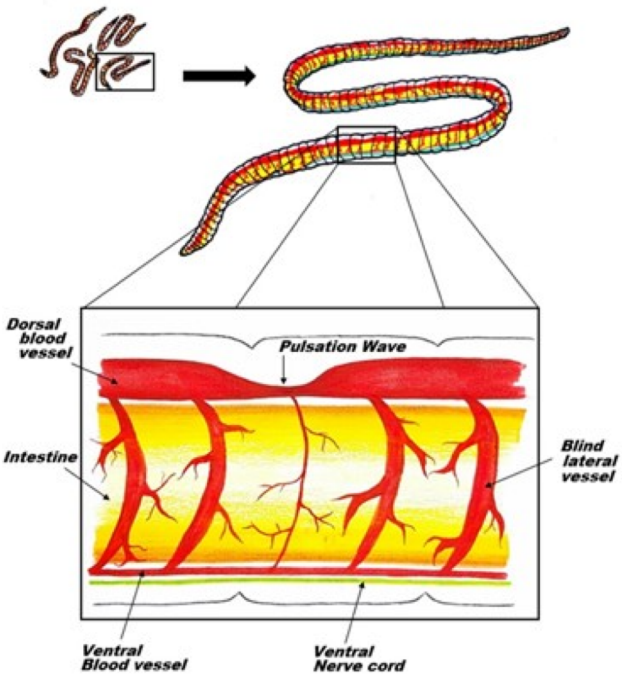


Fig.1. A lateral cross section image of a closed circulatory system found in blackworms. Included in this image are major blood vessels and the internal anatomy. The pulsation rates can be determined by counting the pulsation waves at one location on the dorsal blood vessel. Image created by Sophie Kim.

This investigative laboratory, designed for a college freshman general biology course, takes a fresh look at a standard blackworm laboratory activity that was first published in 1999 (Lesiuk & Drewes) and again in 2006 (Bohrer, 2006). Lesiuk and Drewes describe how blackworms can be used as a model system to demonstrate the effects of nicotine and caffeine on blood vessel pulsation and explain how to make the blackworm viewing chambers. Bohrer provides a more in-depth background as to how the Lesiuk and Drewes preparation can be incorporated into the curriculum, by including timelines, materials, methods, and a suggested grading rubric. We have developed two new approaches for viewing the blackworms under the microscope (*Worm Viewing Chambers* under Procedures) and have added three drugs to those from the original publications. This activity was done over three weeks. Week 1 (Wk 1) instructed the students on blackworm handling and behavior, as well as determining the pulsation rate under control and experimental conditions (varying caffeine concentration). Wk1 served as the practice week for procedures done in week 2. Week 2 (Wk 2) was inquiry, with each pair of students exposing their worms to one of three new treatments (cinnamon, celery seed extract, and valerian root). Wk 2 served as the application week. Both Wk 1 and Wk 2 stressed laboratory skills, including scientific inquiry, data collection, and microscopy. Scientific inquiry included the students becoming skilled at stating a hypothesis (Wk 1), determining the controls (Wks 1 & 2), calculating drug concentrations (Wks 1 & 2), determining results (Wks 1 & 2), and in Week 3, graphing and data analysis used in reaching their conclusions. The laboratory protocol can be easily altered for an advanced biology activity by involving more chemicals, examining reaction to a variety of stimuli (i.e. temperature), and regeneration rates.

PROCEDURES

Blackworm Care

Blackworms were obtained from Carolina Biological and kept in a small tank containing approximately 2 inches of aerated spring water and strips of brown paper towel. They were fed fish food flakes every two weeks. When not in use, the tank was placed in the dark.

Worm Viewing Chambers

We came up with two very distinct, but easy to use viewing chambers for the blackworms. In the original publication (Lesiuk & Drewes, 1999), a viewing chamber was made by using six layers of Parafilm bonded onto a microscope slide using heat. The trough, which held the blackworm, was made by cutting the Parafilm with a razor blade. Using a 3D printer, we designed a plastic slide with the trough embedded, and simply glued this onto a microscope slide (Figs. 2 & 3). A Makerbot Replicator 2 and the Solidworks 3D CAD software program was used to generate the viewing slide. The dimensions for the viewing slide was 7.5 cm long x 2.5 cm wide x 0.2 cm high, and the trough slot embedded within the slide was 4 cm long x 0.2 cm wide x 0.2 cm high. Once these dimensions were entered into the software program, it was exported into

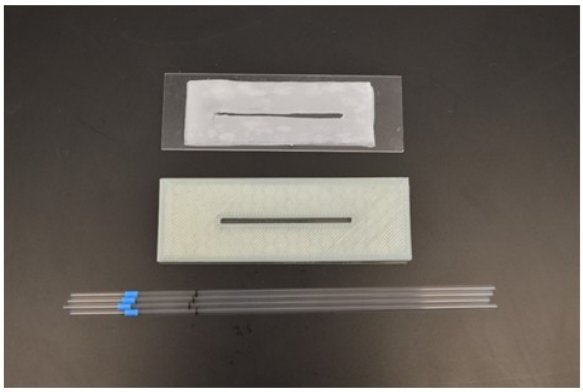


Fig. 2. At the top is the traditional parafilm made trough as described in Lesiuk and Drewes (1999). In the middle is our 3D made trough and at the bottom are 100 µl capillary tubes. All can be used for viewing blackworms under the microscope. Photo taken by N.L. Elwess.

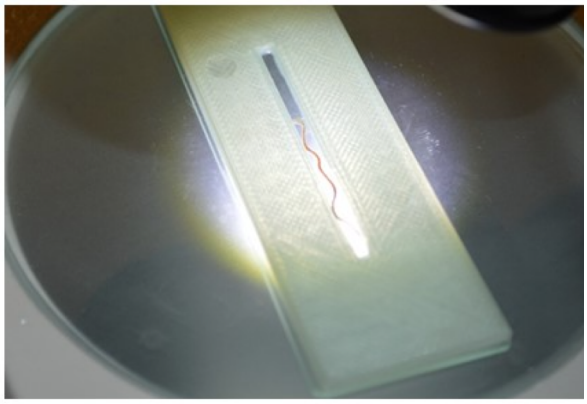
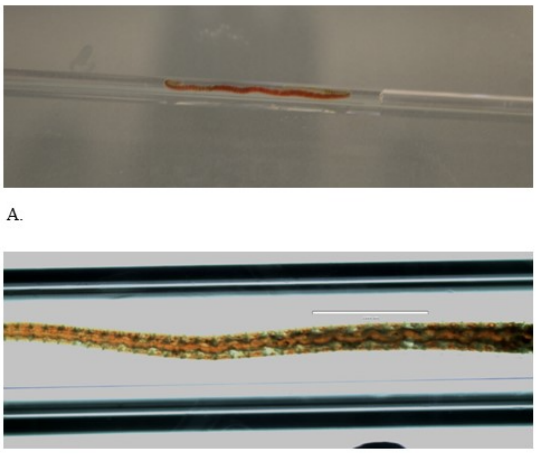


Fig.3. On the microscope slide is a blackworm in a 3D printer made trough. Photo taken by N.L. Elwess.

Makerbot's software. Our second viewing chamber was a 100 µl glass capillary tube (Figs. 2 & 4) with a diameter of 1.35 mm. A worm can be easily transferred with a plastic Pasteur pipette into the capillary tube (Fig. 4). The capillary tube was then placed directly onto the microscope stage, where it could be easily rotated to obtain the best view of the DBV.

Prior to Starting this Laboratory Activity

The week prior to starting this laboratory activity, students were given a pre-lab assignment that was due the first day of this lab. Included in this pre-lab assignment were questions students needed to answer based on their reading of the introductory material in their laboratory manual. These questions covered their knowledge of why blackworms would make a good model organism for determining pulsation rates, parts of a dissecting microscope, and asked the students to provide a hypothesis for the effect of caffeine on blood vessel pulsation. The laboratory activity prior to this experiment introduced students to the nature of science, scientific inquiry, and basic experimental design terminology. This activity we felt was a good follow up to build on their understanding of those concepts. This article focuses on improvements made for the viewing of the blackworms and some suggestions for new experimental approaches to those previously published (Lesiuk & Drewes, 1999; Bohrer, 2006). Bohrer (2006) does an excellent job describing the lab set-up, student learning objectives, materials needed, and a suggested grading rubric.



A.

B.

Fig. 4. A. Image of blackworm as seen in capillary tube under no magnification B. Image of blackworm as seen in capillary tube under 40X magnification Scale bar represents 1000 mm. Photos taken by N.l. Elwess.

Week 1

During Wk 1, the students became familiar with handling the blackworms and determining the basal pulsation rate. Each pair of students selected three blackworms from the tank and transferred them, using a plastic Pasteur pipette, into three separate petri dishes. Once students became comfortable with handling the blackworms, they started counting the DBV pulsation rate for each blackworm; each pulsation rate was done in triplicate. Students viewed the blackworms under a dissecting microscope at 20x magnification. Four different mM concentrations of caffeine were used (0.1, 1, 5, and 10). Working in pairs, students counted and recorded the basal pulse rate for each worm, this served as a control, and then tested one of the four caffeine concentrations. Students made 30 mL of the test solution by diluting a 20mM caffeine stock solution. Each worm was placed in 10mL of test solution. A blackworm was exposed to the test solution for 15 minutes prior to counting the pulsation rate. Students recorded and shared their data using Table 1.

Table 1: Effect of Caffeine on Pulsation Rate in *Lumbriculus variegatus*								
	Control (bpm)	0.1mM (bpm)	Control (bpm)	1.0mM (bpm)	Control (bpm)	5.0mM (bpm)	Control (bpm)	10.0mM (bpm)
Worm 1								
Trial 1								
Trial 2								
Trial 3								
Average								
Worm 2								
Trial 1								
Trial 2								
Trial 3								
Average								
Worm 3								
Trial 1								
Trial 2								
Trial 3								
Average								

Week 2

Three different compounds were used to determine if they had an influence on pulsation rate. All lab groups repeated the same approach as in Wk 1, but in Wk 2 instead of testing caffeine, they tested two concentrations (0.1 mg/mL and 1.0 mg/mL) of Cinnamon, Celery seed extract, or Valerian root. Three worms were observed at each concentration and each blackworm was placed in a single petri dish. Cinnamon, a spice widely used in traditional medicine, has been linked to a reduction in cardiovascular disease due to its effects on excitability (Alvarez-Collazo et al., 2014). Celery seed extract, which has been used in the Eastern world for thousands of years, is a diuretic used in the treatment of high blood pressure (Moghadam, et al., 2013). Valerian root is an herbal supplement used to reduce blood pressure and prevent arrhythmias (Chen et al., 2015). Students calculated the appropriate dilution needed to make 30 mL of test solution from the commercially purchased stock solutions of cinnamon (905.7 mg/mL), celery root extract (870 mg/mL), and valerian root (1000mg/mL). Students recorded and shared their data using Table 2.

Week 3

Each student generated graphs of the average pulsation rates for each experiment

Table 2: Effect of _____ on Pulsation Rate in *Lumbriculus variegatus*				
	Control (bpm)	0.1 mg/mL (bpm)	Control (bpm)	1.0 mg/mL (bpm)
Worm 1				
Trial 1				
Trial 2				
Trial 3				
Average				
Worm 2				
Trial 1				
Trial 2				
Trial 3				
Worm 3				
Trial 1				
Trial 2				
Trial 3				
Average				

using Microsoft excel (Figs. 5-7). Using the graphed data, the students determined if their initial hypotheses were supported or refuted and then wrote their conclusions.

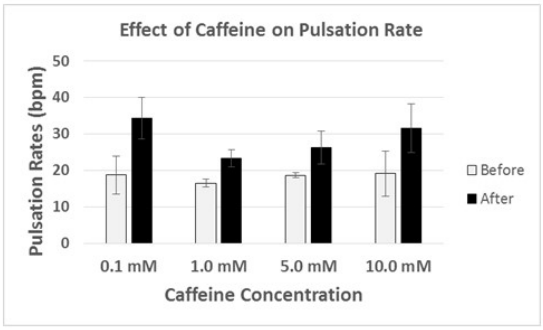


Fig. 5. An example of a student generated graph showing the results of the effect of caffeine treatment on pulsation rates in blackworms. There was a significant difference at 0.1 mM, 1.0 mM, and 5.0 mM concentrations of caffeine. Each of those experimental conditions had a high pulsation rate over the control.

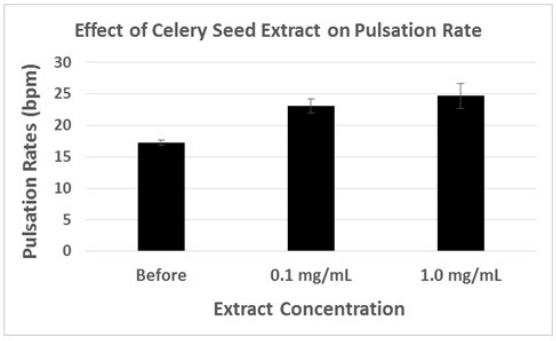


Fig. 6. An example of a student generated graph showing the results of the effect of celery extract on pulsation rates in blackworms. At both concentrations (0.1 mg/mL, 1.0 mg/mL) there was a significant increase in pulsation rates over that of the control.

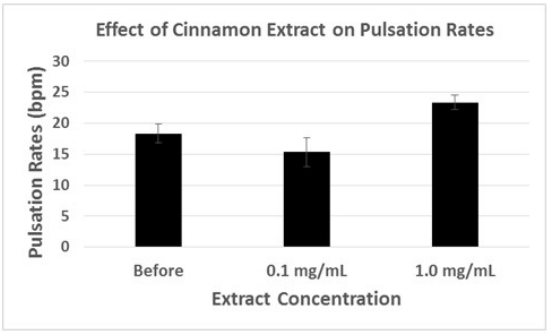


Fig. 7. An example of a student generated graph showing the results of the effect of cinnamon on pulsation rates in blackworms. At the 1.0 mg/mL there was a significant increase in pulsation rates over that of the control, while the 0.1 mg/mL concentration did not show any difference in pulsation rates.

RESULTS AND DISCUSSION

Overall, the updated worm viewing chambers used in this lab allowed students to better manipulate the worms under the microscope for optimal viewing of vessel pulsation. The 3D generated viewing chamber worked well for viewing larger blackworms, and the capillary tubes worked well with any size blackworm. The second week of the activity, where a novel compound was tested for its effect on pulsation, provided students the opportunity to compare treatment groups and perform statistical analyses. The inclusion of novel compounds also required the students to perform literature searches for candidate molecules and gave them the opportunity to develop testable hypotheses and make predictions based on their findings. As an experimental modification, students could also research and select the novel compound that they will assess, and design their own experiments, thereby introducing another component of scientific inquiry. They could also assess the effect of their compound on additional parameters, including blackworm behavior, reflex activity, or regeneration.

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