The Journal of Undergraduate Neuroscience Education (JUNE), Spring 2016, 14(2):R25-R31

TECHNICAL REPORT
Running Wheel for Earthworms

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We describe the construction and use of a running wheel responsive to the movement of the earthworm. The wheel employs readily available, inexpensive components and is easily constructed. Movement of the wheel can be monitored visually or via standard behavioral laboratory computer interfaces. Examples of data are presented, and possibilities for use in the teaching classroom are discussed.

Key words: earthworm; locomotion; running wheel; invertebrate; classroom

The undergraduate neuroscience or psychology curriculum is enhanced by courses that emphasize the collection of behavioral data (Wiertelak and Ramirez, 2008); research experience is rated as essential to admission to and success in graduate neuroscience programs (Boitano and Seyal, 2001). The cost of acquiring and maintaining vertebrate organisms, and the increased time and paperwork necessitated by Institutional Animal Care and Use Committee oversight, have reduced the use of vertebrate organisms for this purpose. At our college, for example, students in our Research in Behavioral Neuroscience course used to propose individual projects with rats, within an "envelope" of possible manipulations that had prior IACUC approval. When the IACUC insisted that every project be individually reviewed, the increase in time necessary for an initial review and likely revisions made this approach unfeasible within the time constraints of a single semester. Furthermore, the cost of rats meant that only one or two of the students' projects would be selected for group completion; disappointment abounded when students realized that the projects they had proposed and in which they were heavily invested would not be done.

These bureaucratic and financial considerations prompted one of us (WJW) to consider alternatives. Abramson et al. (2011) and others have addressed the use of invertebrates in teaching about behavior. Kladt et al. (2010) and Shannon et al. (2014) discuss the merits of using earthworms for the teaching of neurophysiology. Weighing the benefits, we decided to bring earthworms into the student laboratory in place of rats. Ever since Darwin's final book (1881) examining earthworms, comparative psychologists have considered the behavioral and mental capacities of these organisms. Many well-controlled studies of the earthworm's behavior have been conducted in the past century, notably beginning with Yerkes' (1912) study examining the ability of *Allolobophora foetida* (now *Eisenia foetida*) to learn a T-maze (see Wilson, 2010 for an annotated bibliography). Yerkes' oft-cited study in fact reported the behavior of a single worm; nonetheless it served as the basis for many more T-maze studies in the worm. Rosenkoetter and Boice (1975) demonstrated, however, that the earthworm's ability to learn a T-maze was in fact not learning at all, but instead involved responses to chemical cues released by the worm on prior trials. Since 1975 most studies of learning in earthworms have examined Pavlovian rather than instrumental learning.

Earthworms are inexpensive—typically costing on the order of 25¢ each—so much so that the price of one or two rats can provide enough earthworms for all students in a class to complete their individually proposed projects. Earthworms are readily available in most areas; in our region the large earthworm *Lumbricus terrestris* can be purchased from bait shops or convenience stores for use as fishing bait. Maintenance of the worms is simple; they will survive for weeks as received from the shop if they are refrigerated, or longer if placed in a medium of peat moss or coconut coir with some ground oatmeal and cornmeal added. We have maintained a reproducing colony of smaller "compost" worms (*Eisenia hortensis*) for years at room temperature in a medium of coconut coir, peat moss, and shredded paper by adding vegetable scraps weekly (many resources that address earthworm husbandry are available, e.g., www.earthwormworks.com; www.theamphibian.co.uk; Lowe and Butt, 2005; Spencer and Spencer, 2006).

IACUC approval is not required for studies with earthworms, eliminating a nearly insurmountable delay in the time-constrained teaching laboratory. This is not to say that the work done is free from ethical or humane constraints; rather those constraints can be taught and imposed by the instructor without committee approval. In our course students propose their earthworm projects by completing our standard IACUC form, which is then reviewed by the instructor rather than by the committee. Students thus come to appreciate the need for approval and the steps necessary to gain that approval, without the delay imposed by committee review.

Yerkes (1912) studied a small "compost" worm; most behavioral studies since then have used *Lumbricus*. *Lumbricus* is a large worm, ~3–10 g. This facilitates the automation of behavioral measurements; most previous work with earthworms involved visual observation of responses, a practice that invariably raises concern about objectivity and unintentional observer bias. After trying several methods of automation (modified drinkometer circuits, Wilson and Renaud, 2012; infrared beams across which the worm crawls, even a "wobble wheel," Wilson, et al., 2012) we settled on the tried and true running wheel. Such wheels are commonly used in studies of rodent circadian activity, and have been successfully employed with invertebrates, including earthworms (crab: Darnell, et al., 2008; cockroach: Brady, 1982; or see Backyardbrains.com for a commercially-available cockroach wheel at backyardbrains.com/products/ CockroachWheel; earthworm: Burns et al., 2009; Marian and Abramson, 1982; McManus and Wyers, 1978). A rodent wheel typically counts each revolution of the wheel. This is not sufficient resolution for the detection of movement in the earthworm, which will take 10 min or longer to complete a single full turn of the wheel. We have designed a wheel such that infrared beams and a quadrature disk allow fractional movement of the wheel to be recorded. A video of *E. hortensis* in a running wheel is available (http://youtu.be/NNiPs16q4vg); this shows six hrs of video condensed into 1 min. It also illustrates a problem with lighter worms - at about the 50-s point you can see the wheel fail to respond to the worm after it reverses direction; the worm crawls counter-clockwise up and around a now non-turning wheel. The heavier *Lumbricus* generally negates this problem.

We describe the development of our running wheel, and offer suggestions for its use in the teaching laboratory.

CONSTRAINT

Earlier efforts to construct a running wheel for an earthworm addressed the problem of constraining the worm by placing it in a tube. We followed the same approach. A 1/2-in o.d., 3/8-in i.d. clear vinyl tube houses the worm while it is being studied. We moisten the inside of the tube with water, often add some ground oatmeal and cornmeal (about 0.5 g), then insert the worm. Although this might seem like a difficult task, it is remarkably easy to accomplish. Worms are thigmotaxic (i.e., they keep their body in contact with the substrate); if the worm is held above the opening of the tube and its head is guided into the opening, it will usually allow itself to be lowered into the tube, even "crawling" in to assist the investigator. In a brightly lighted room, wrapping one's hand around the tube darkens the interior and facilitates the process because the worm will tend to crawl into this darkened space. Once the worm is fully inside the tube, the ends of the tube are brought together and secured with a piece of transparent tape wrapped around the joint.

CIRCULARITY

Once the worm is securely within the constraining tube, the tube must be mounted vertically in a circular manner (a noncircular tube would result in weight imbalances that would impede free turning). Others have mounted the tube on a reel-to-reel recording tape spool (Burns et al., 2009) or have created circular indentations in two pieces of plastic that when conjoined formed a circular channel (Marian and Abramson, 1982; McManus and Wyers, 1978). The tape spool approach seemed simplest and most economical, but such spools are not easily obtained. Instead we use a plastic flying disk (e.g., Frisbee brand); the tube holding the worm is just long enough to fit snugly inside the rim of the disk. We secure the tube with two pieces of transparent tape. Flying disks are readily available, inexpensive, and nicely balanced.

AXLE

The wheel must be mounted in a manner that allows it to turn freely—especially important because the earthworm weighs very little. Too much friction and the wheel will not rotate, allowing the worm to crawl up and around the wheel. In our first attempt we glued a T-nut to the center of the disk, and placed this loosely over a bolt that was mounted to a piece of wood. This worked much of the time, but the threads within the T-nut engaged the threads in the smaller bolt and created problematic friction.

Our next attempt used bearings designed for use in skateboards. These bearings have a central cylinder surrounded by ball bearings on which an outer cylinder is mounted. We placed the T-nut into the central cylinder, and secured it with a bolt inserted into the central cylinder from the other side. The bearing was then placed into a form-fitting hole cut into a piece of wood, and was secured with a screw and washer at the top. This bearing allowed the wheel to turn much more freely than did our earlier bolt axle. Over time, though, the weight of the wheel pulling down on one side of the bearing created wear resulting in the wheel drooping; skate bearings are designed to support weight pushing down on the central cylinder, not weight pulling more on one side than the other.

Our final approach to mounting the wheel took advantage of the normal use of the skate bearing. We secured a skateboard wheel in a form-fitting hole in a piece of wood; in normal use the skateboard wheel has two bearings, one on each side, through which an axle extends. We extended a bolt from the T-nut through both bearings and secured it with a nut. With two bearings the axle extending from the disk was better supported and droop was prevented.

RECORDING MOVEMENT

Commercial rodent running wheels typically have counters affixed that record each full revolution of the wheel. Because earthworms move more slowly than a typical rodent we needed a method of recording incremental movement of the wheel. Burns et al. (2009) did this by mounting the tape-spool wheel on the shaft of a rotary motion sensor; the output of this sensor could be read to provide information about movement of the wheel. Such rotary sensors are expensive (> $150 US) and their shafts are typically not designed to support the weight of the running wheel. Our first effort involved infrared (IR) beams passing through small holes drilled in the plastic disk and detected by sensitive photodarlingtons. We have previously shown that such simple electronic circuits can function as switches when connected directly to Med Associates or Colbourn interfaces (Wilson, 2004). Aligning the IR beams proved difficult with our initial bolt axle because of "play" in the movement of the wheel which was simply suspended on the bolt; as well as with our one-bearing mount because of droop.

We turned to magnetic reed switches as a means of recording. Eight permanent rare earth magnets were glued to the plastic disk, and three reed switches were mounted to the support, offset from each other such that a given magnet would affect each of the three switches in turn. In theory this allowed movements equivalent to 1/24th of the circumference of the running wheel to be recorded; in practice, though, we detected approximately 36 counts for each revolution. Reed switches open and close twice each time a magnet passes near them, and the droop of the wheel resulted in irregularities in magnet detection. We could reliably record movement, but each count reflected a differing and really unknowable amount of movement. For this reason, and because of studies that suggested that some invertebrates, maybe including earthworms, are sensitive to magnetic fields, we stopped using magnets (Bennett, 1974; Vidal-Gadea et al., 2015).

We have now settled on what appears to be a much more reliable means of recording movement of the running wheels. We mount a quadrature disk on the axle of the wheel that extends out the back of the mount, and record the rotation of this disk with two infrared sensors. A quadrature disk can take several forms; ours is simply a thin opaque plastic disk with 32 slots cut out around its circumference. The slots allow the IR beams to pass through, striking the photodarlingtons. The two IR detectors are offset from each other such that one or the other, both, or neither can be activated at any point in time. If each is considered a switch, this arrangement results in the following sequence of switch closures: 0-0, 1-0, 1-1, 1-0. When recorded by a computer (e.g., using Med-PC software), the direction of the rotation can be ascertained.

An alternative to the automated detection of movement is to use video recording equipment or time-lapse photography to record the position of the wheel. This is facilitated if a black and white pattern is mounted on the disk such that changes in position are readily apparent. We have used a USB video camera and free software (streamer by Gerd Knorr, available in most Linux distributions; similar applications, e.g., SkyStudioPro, are available for other operating systems) to record images of the wheel every 20 sec over the course of 1 or 2 days. Movement of the wheel can then be scored by students, either from the still images or after they have been converted to a video. Finally, students could monitor movement of the wheel in real time. We favor the computerized monitoring of the wheel because in our studies of instrumental learning (e.g., escape and punishment) the presentation of stimuli must be made contingent on the worm's behavior, and our computer software that monitors movement can also control the stimuli. Students observing movement in real time could achieve the same thing. If response-contingent control of stimuli is not necessary, then analysis of wheel movement from the video recording after the fact would work just as well.

COMPLETE DESIGN

Details of construction are provided in Figure 1. Each wheel consists of a plastic flying disk mounted on skate wheel bearings; a clear plastic tube around the wheel's circumference houses the worm. IR beams record movement of the wheel via rotation of the quadrature disk. The wheel is balanced by adjusting the positions of two binder clips placed on the edge of the disk such that the wheel, when spun gently, stops in different orientations each time.

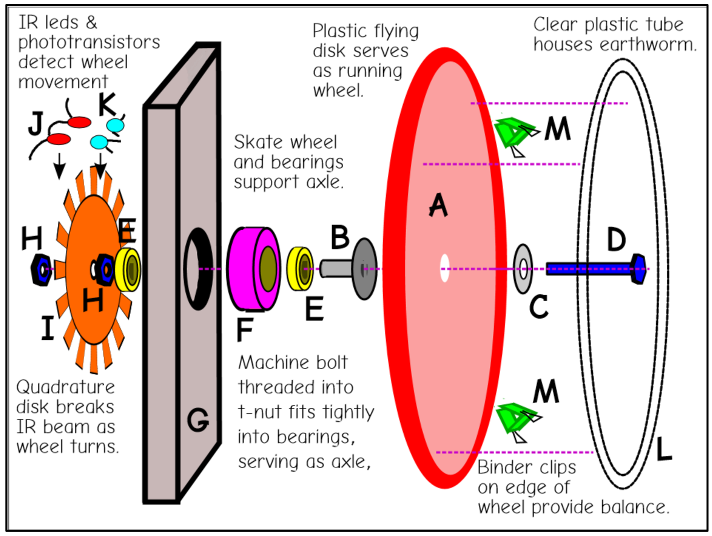

Figure 1. The running wheel consists of a flying plastic disk 27.5 cm in diameter (A) with a hole drilled in its center and a 5/16 –18 x5/8 T-nut (B) glued to its exterior around the hole. A fender washer (C) is placed opposite the T-nut base and a 5/16–18 x 2.5-in machine bolt (D) is inserted through the washer and threaded tightly into the T-nut. The T-nut-bolt assembly is inserted into a skate wheel (F) (complete with bearings, E, on each side), that is flush-mounted into a hole drilled through a 3/4-in pine board (G); the wheel is secured by a screw inserted through the edge of the mounting board and into the wheel itself (not shown). A nut (H) is tightened onto the machine bolt on the back of the skate wheel, securing it to the center portion of the bearing. A quadrature disk (I), laser-cut from 1/8-in plastic, is 120 mm in diameter, with 32 evenly spaced 5.624° cut-out gaps. This disk is mounted on the bolt and secured with an additional nut (H). Infrared LEDs (J) (Fairchild QED123) and photodarlingtons (K) (Optek OP830WSL) are mounted in a bracket (not shown) that straddles the quadrature disk; these are positioned such that they are sequentially activated in the pattern 00–01–11–10.... In theory this allows each movement of 1/128th circumference of the wheel to be detected; because we program the computer to count only when a pattern differs from the prior two (to prevent back-and-forth rocking of the wheel from being counted) in practice we count movements equivalent to 3/128th of the circumference. The earthworm is inserted into a piece of vinyl tubing (L)(1/2 -in o.d., 3/8 -in i.d.) cut to fit snugly inside the rim of the disk, and secured there with transparent tape. Two spring-loaded binder clips (M) (0.5 in, 2.75 g, "micro", Office Max, Naperville, IL USA) are used to balance the wheel (with the tube in place); this is done before the worm is inserted and is repeated weekly.

Eight wheels are contained in individual compartments in a plastic utility cabinet with four shelves, each divided by a Styrofoam panel (see Figure 2). This cabinet is in a sound-attenuated chamber in a dark room below ground level, and is maintained at approximately 20° C. Sessions are conducted with the doors of the utility cabinet closed, so that wheels are in the dark, unless a photographic or video record is being made; in that case the doors are open and a dim red light illuminates the wheels (earthworms cannot sense red light, Walton, 1927).

Two white 31-mm (1.25-in) 12-V festoon dome light LED bulbs (3528 12-SMD, 72 lumen, generic) wired in series are positioned 7.6 cm out from the lower portion of the wheel, where the earthworm would be located. These are powered directly by the 28-V provided by the Med Associates interface. Although this is more voltage than the LEDs require (i.e., 12 V x 2 = 24 V when wired in series); they can tolerate the excessive voltage.

Data presented below are from worms housed in a peat moss and coconut coir medium within an opaque plastic tub in a chest freezer modified to remain at 13°C.

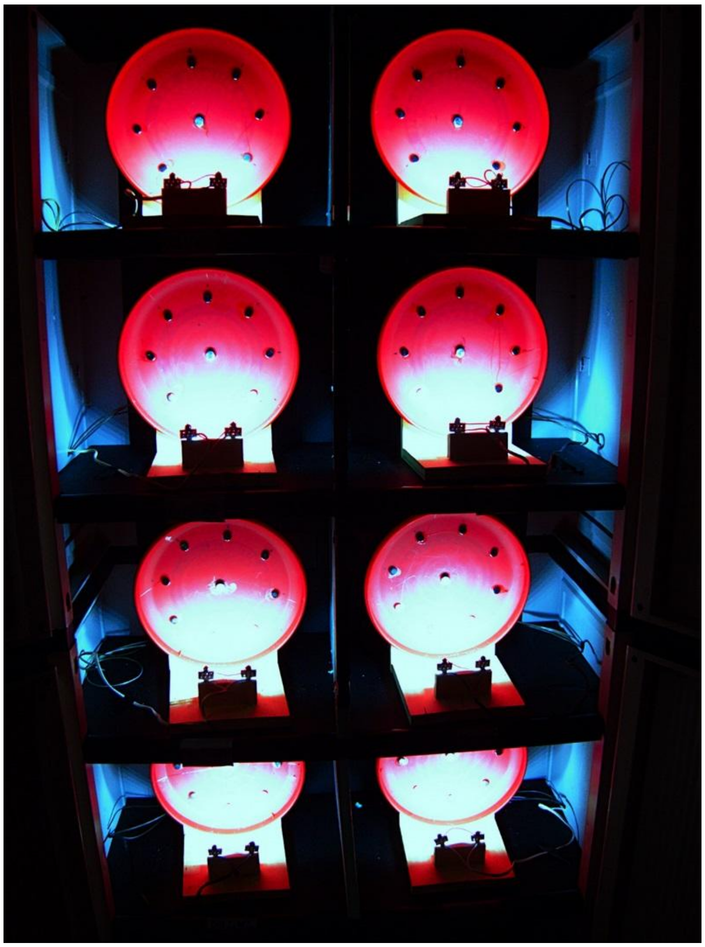

Figure 2. Earthworm Running Wheels in Enclosure, Eight running wheels housed in plastic utility cabinet. When doors are closed each wheel is in the dark, and is isolated visually from the other wheels.

TYPICAL RECORDINGS

We present data representative of baseline activity of earthworms in the dark, and of the worm's response to a bright light.

Figure 3 shows the individual responses of 8 *Lumbricus* over a 24-hr period. Each worm's responses were converted to z-scores for each 10-min interval to facilitate comparisons across worms:

$$z = \frac{(R - M)}{\sigma} \qquad (1)$$

where *R* = a worm's responses in a 10-min interval, *M* = a worm's mean number of responses across all of its 10-min intervals, and *σ* = the standard deviation of the worm's responses across all intervals. Summary data for each worm appear in Table 1. It is clear that there are individual differences in baseline responsiveness of the earthworms.

Worm	Weight (g)	Mean Rs/10-min	σ
1	8.64	1.22	2.23
2	5.90	0.13	0.48
3	6.18	0.49	1.40
4	7.95	6.12	7.10
5	5.98	1.36	2.48
6	3.96	0.09	0.33
7	3.57	2.51	3.54
8	5.87	0.09	0.57

Table 1. Data related to the eight worms whose standardized responses are presented in Figure 3.

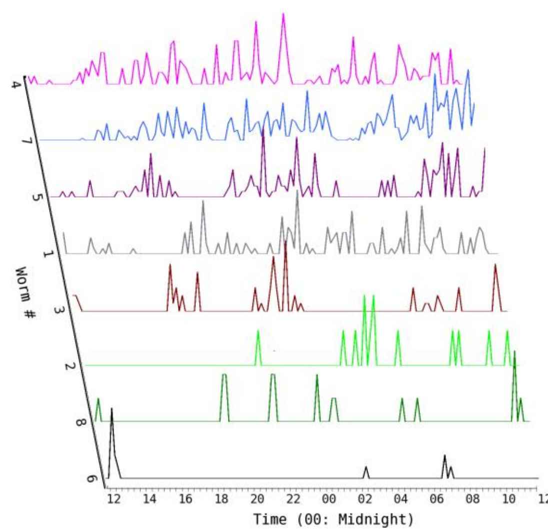

Figure 3. Movement of eight earthworms over a 24-hr period, in 10-min blocks. Worms were housed in darkness and the wheels were dark throughout the session. Movement for each worm was standardized as z-scores based on the mean and standard deviation across the 24-hr session. Variability across worms is apparent.

Mean responses across all eight worms appear in Figure 4. Nothing can be said about circadian rhythms in the worms' movement on the basis of a 24-hr record, although it appears that activity declined across worms at about 0400. We leave it to others to assess the earthworm's circadian rhythm, and to determine if the decreased movement at 0400 is real or artefactual.

The data in Table 1 indicate no correlation between the worm's weight and its movement (r = .36, p = .37). For 94 other worms, run for 60 min, there was also no relationship between weight and responses recorded (r = .04, p = .70), suggesting that the wheel is not more sensitive to movement of heavier worms.

The locomotor response triggered by a bright light is apparent in Figure 5. Twelve worms were placed in the wheels in the dark for 24 hr, and bright white lights were illuminated 12 times for 10-min intervals. Worms crawled far more when the light was on than they did in the dark. This reflects the worm's negative phototropism, no doubt an adaptive mechanism to help it avoid detection by predators and the drying effect of being above ground and exposed to sunlight. We have used this apparent aversive response to bright light (Walton, 1927) in our studies of escape behavior (Wilson et al., 2013, 2014).

APPLICATIONS

We have examined both escape (Wilson et al., 2013) and punishment (unpublished data) paradigms using the running wheels, with bright light serving as the negative reinforcer or punisher; earthworms' behavior is sensitive to both paradigms. A current project in our laboratory is using the wheels to study habituation of the worm's response to bright light. Another project is assessing the effect of MK-801, an NMDA receptor blocker suggested to interfere with memory consolidation in vertebrates, on escape learning. Our running wheel in essence provides a "manipulandum" that is responsive to the worm's behavior, thus facilitating studies of instrumental learning.

Our worms survive and remain apparently healthy in the wheels for sessions of 48-hr duration (we add water and food as described above in Constraint); survival for longer periods should be expected. Thus the wheels might be used for a careful examination of circadian rhythms.

In addition to being sensitive to light, earthworms can sense vibration (Ratner and Miller, 1959) and chemicals (Laverack, 1960, 1963), so stimuli other than the bright lights that we have described could be employed. One student in our laboratory attached automotive relays to the support of the running wheel and cycled them on and off at varying frequencies to created vibratory stimuli. Chemical "odorants" could be used to create distinctive environments within the wheels for studies of conditioned place aversion (e.g., rose oil wheel associated with frequent bright lights). And of course, the effects of drug or neural manipulations of the worms on their general locomotion or learning could be studied.

Because of the low cost of the wheels and of earthworms, each student in a laboratory course examining animal learning or behavior could design and conduct his or her individual study. Working on a project of one's own design adds a sense of ownership that makes the experience more meaningful and therefore more engaging and rewarding. We hope that those who adopt our wheel design will share their experiences (both good and bad) with us.

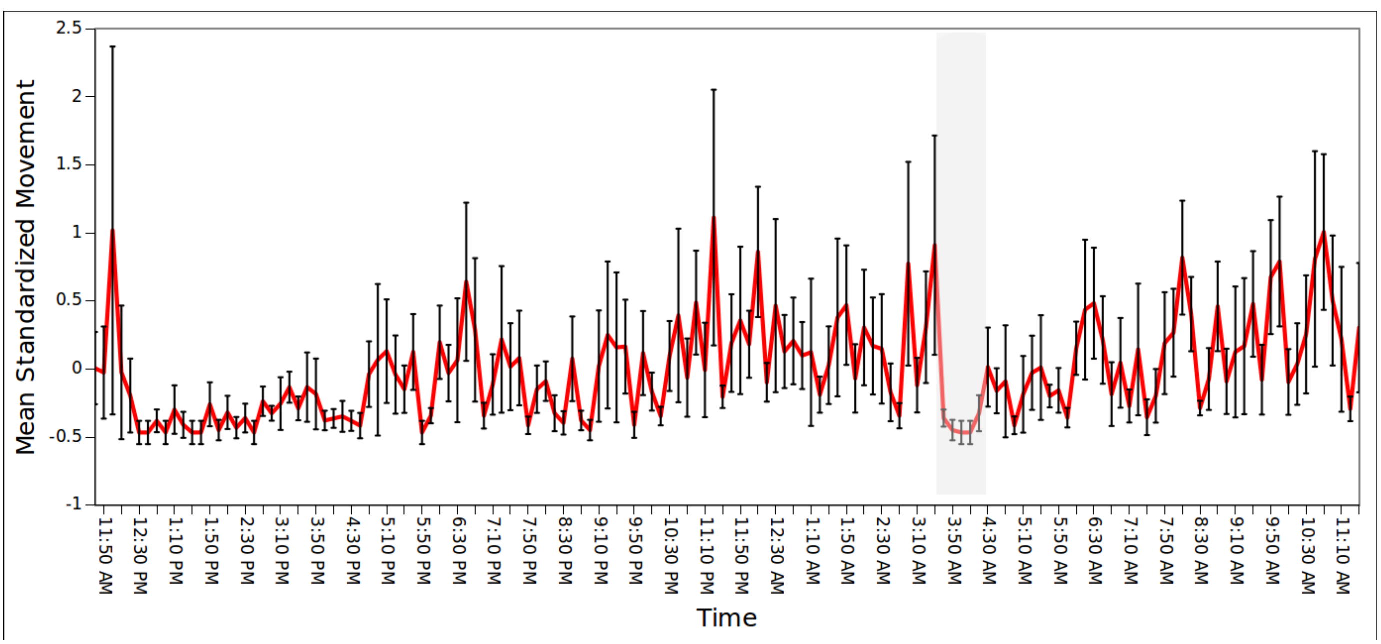

Figure 4. Mean standardized movement of eight earthworms over a 24-hr period. Worms were housed in darkness and the wheels were dark throughout the session. Responding declined across all worms at about 0400 (grey bar). Perhaps an enterprising student can determine if this is characteristic of circadian activity in *Lumbricus*.

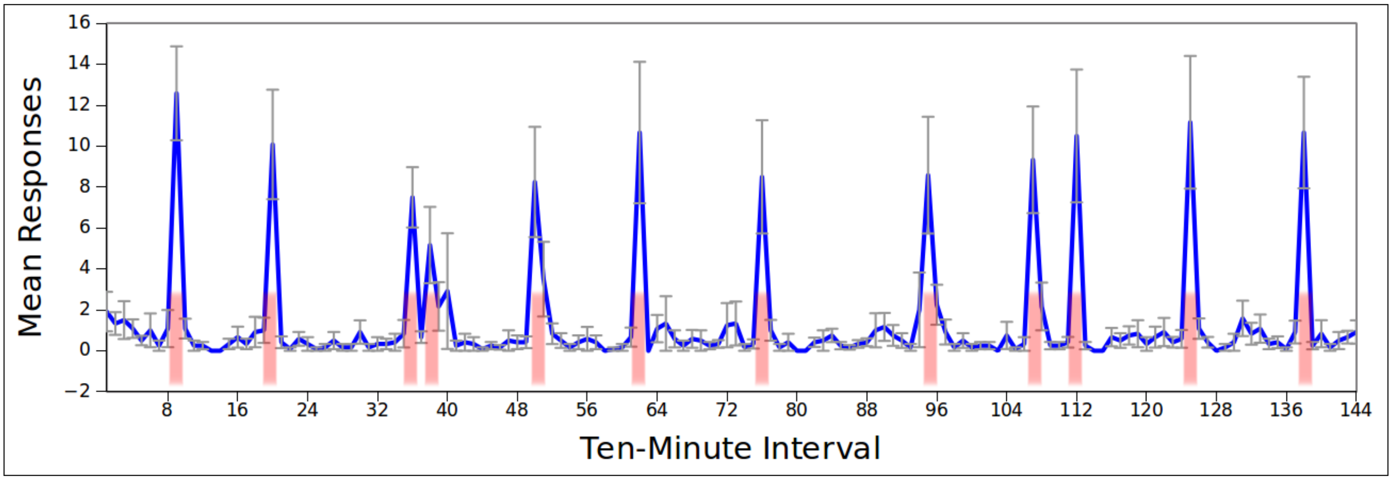

Figure 5. Responses to bright light of 12 worms over a 24-hr period. Wheels were in the dark; bright white lights were turned on 12 times for 10 min (pink bars). During the bright light the worms moved; worms responded very little except when the white light was presented. Two periods of light in close succession (Intervals 36 and 38) seemed to yield longer-lasting movement, extending to Interval 40 when no light was presented. There was no contingency between movement and light onset or offset in this study.

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Received January 08, 2016; revised March 08, 2016; accepted March 15, 2016.

This work was supported by Albion College's Foundation for Undergraduate Research, Scholarship, and Creative Activity; Neuroscience Program; and Dept. of Psychological Science. BAJ is currently an undergraduate student. We thank the editor and two anonymous reviewers for useful suggestions. We appreciate the prior contributions of former students whose work contributed to the development of this final version of the wheel, notably Ashley Glenn, Emily Morlock, Holly Paxton, Elizabeth Renaud, and Megan Wickens. We gratefully acknowledge the assistance of J Brooks Zurn who made the quadrature disks for us.

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Appendix A. Required parts and their estimated costs.

Qty	Description	Source	Each	Cost [1] (approx.)
1	Flying disc - e.g., Discraft 175 gram Ultra Star Sport Disc	Amazon	8.99	8.99
1	Skate wheel assembly (with bearings) – e.g., Big Boy 52mm Blank Skateboard Wheels + ABEC 7 Bearings Spacers (Purple)	Amazon	3.25	3.25
1	Pine board - 3/4 x 8 x >12 in – length arbitrary [2]	Lumber store	1.39	1.39
1	T-nut - 5/16 –18 x 5/8 in.	Hardware store	1.39	1.39
1	Fender washer	Hardware store	0.59	0.59
1	Machine bolt - 5/16–18 x 2.5-in	Hardware store	0.89	0.89
2	Hex nut – 5/16-in	Hardware store	0.29	0.58
1	Vinyl tubing - 1/2 -in o.d., 3/8 -in i.d., cut to fit snugly within rim of flying disc	Hardware store	0.89/ft	2.50
1	Quadrature disc – see text for description	Custom	???	???
2	Infrared emitting diodes – e.g., Fairchild QED123	Newark.com	0.52	1.04
2	Photodarlingtons – e.g., Optek OP830WSL	Newark.com	2.67	5.34
2	Spring-loaded binder clips - 0.5 in, 2.75 g, "micro" # 1378873	OfficeMax.com	3.99/100	0.08
1	Bracket for mounting IEDs and sensors[3]	Custom	1.00	1.00
1	Wood screw to secure skate wheel into board – size as necessary.	Hardware store		0.39
Total	Estimated total cost without quadrature wheel			$27.43

[1] Prices of hardware items will vary depending on source, and on whether purchased in bulk or individually.
[2] We mount wheel in 8-in. piece, and use another 8-in. piece mounted at right angles as the base.
[3] We fashioned U-shaped bracket from three pieces of thin plywood sandwiched together.