Earthworms in Behavioral Neuroscience: Nearly a Century of Studies

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Introduction

YERKES (1912) EXAMINED the ability of earthworms to learn a T-maze. Since then the earthworm has been a subject not only in other T-maze studies, but also in studies of Pavlovian conditioning and circadian rhythms. Its relatively simple nervous system makes the earthworm an ideal candidate for behavioral neuroscience studies; its relatively limited sensory and behavioral repertoire make such studies challenging.

Earthworm Species

EARTHWORMS COMPRISE thousands of different species. One, *Lumbricus terrestris* or the Canadian nightcrawler, has been the focus of most laboratory study, possibly because it is the largest worm readily available in Europe and North America; its length can approach 25 cm. However, *Lumbricus terrestris* is not easy to maintain in a laboratory environment; it lives naturally in a permanent burrow that can be as deep as 1 or 2 m. Even if the proper habitat is created in the laboratory, retrieving the worm for study would be difficult. Two other species, also relatively large, are easily capable of being maintained in a laboratory because they are epigeic — they live in loose leaf litter or burrow very shallowly in loose dirt. These two species, *Eudrilus eugeniae* and *Eisenia hortensis* (respectively the African and European nightcrawlers) are far less common in behavioral studies but are the species with which I am working.

Figure 1: *European (left) and African (right) nightcrawlers.*

Instrumental Learning

INSTRUMENTAL LEARNING WAS FIRST studied by Yerkes using a T-maze. *Allolobophora foetida* (now *Eisenia fetida*) was punished with an electric shock for turning in one direction, and rewarded with access to a dark moist chamber for a turn in the opposite direction. Following his lead, many others used this T-maze procedure with earthworms until 1975, when Roesenkoetter & Boice demonstrated that if a new maze is used for each trial the behavior reverts to chance; worms secrete a chemical when shocked that can guide subsequent behavior. Instrumental learning has rarely been studied in earthworms since 1975. [List of references provided here and in the other sections is not meant to be exhaustive, but is believed to include most of the relevant studies.]

- Arbit, J. (1957). Diurnal cycles and learning in earthworms. *Science*, *126*, 654–655. [T-maze; time-of-day effects]
- Bharucha-Reid, R. P. (1965). Latent learning in the earthworm. *Science*, *123*, 222. [T-maze; latent learning]
- Caldwell, W. E. & Kailan, H. (1955). An investigation of the role of exteroceptive motivation in the behavior of the earth-worm *The Journal of Psychology*, *40*, 133–144. [Complex maze; latent learning]
- Datta, L. G. (1962). Learning in the earthworm, *Lumbricus terrestris*. *The American Journal of Psychology*, *75*, 531–553. [T-maze; performance back to näive levels after 15 days]
- Keshavamurthy, P., & Krishnamoorthy, R. V. (1977). A circadian rhythm in the electrode-avoidance behavior of the earthworms *Megascolex mauritii*, *Pheretima elongata*, and *Perionyx excavatus*. *Behavioral Biology*, *20*, 17–24. [T-apparatus; shock in both arms]
- Kirk, W. E., & Thompson, R. W. (1967). Effects of light, shock, and goal box conditions on runway performance of the earthworm *Lumbricus terrestris*. *The Psychological Record*, *17*, 49–54. [Runway; red v white light; moss v empty goal box]
- McManus, F. E., & Wyers, E. J. (1979). Differentiating ganglionic function in the Earthworm. *Journal of Comparative and Physiological Psychology*, 93, 1136–1144. [T-maze; saline as aversive stimulus; severed connections to ganglia]
- McManus, F. E., & Wyers, E. J. (1979). Olfaction and selective association in the earthworm, *Lumbricus terrestris*. *Behavioral and Neural Biology*, *25*, 29–57. [T-maze; odors, inclination]
- Miller, D. B., & Tallarico, R. B. (1972). Acquisition, extinction, and spontaneous recovery of a positively reinforced approach response in the earthworm, *Lumbricus terrestris*. *The Psychological Record*, *22*, 381–386. [Runway; escape from light into sphagnum moss]

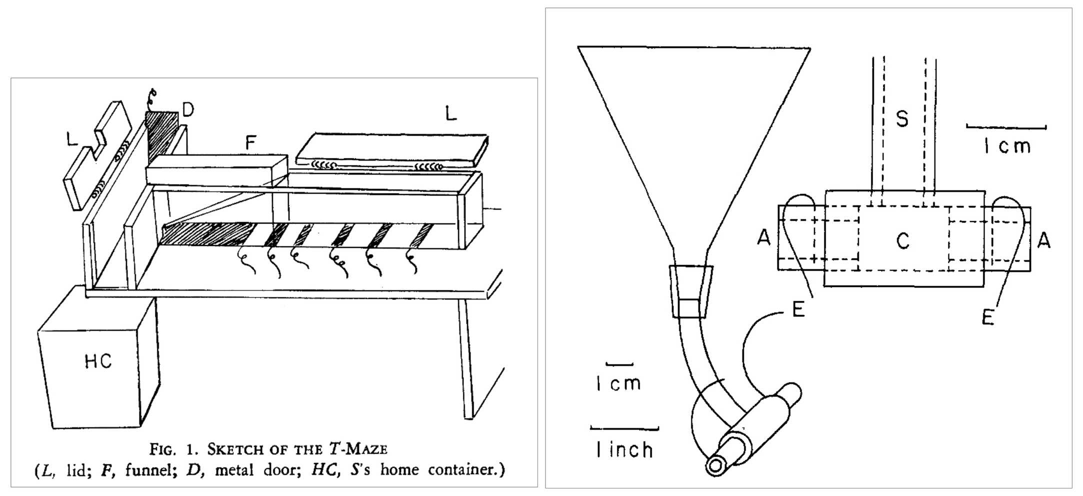

Figure 2: *Datta's (left) and Rosenkoetter's (right) T-Mazes*

- Ressler, R. H., Cialdini, R. B., Ghoca, M. L., Kreist, S. M. (1968) Alarm pheromone in the earthworm *Lumbricus terrestris*. *Science*, *161*, 597–599. [Escape from harvested pheromone]
- Reynierse, J. H. (1968). Effects of temperature and temperature change on earthworm locomotor behaviour. *Animal Behaviour*, *16*, 480–484. [Runway; temperature effects]
- Reynierse, J. H., Halliday, R. A., & Nelson, M. R. (1968). Nonassociative factors inhibiting earthworm straight-alley performance. *Journal of Comparative and Physiological Psychology*, *65*, 160–163. [Runway; role of stimulus change and goal-box floor depth]
- Reynierse, J. H., & Ratner, S. C., (1964). Acquisition and extinction in the earthworm, *Lumbricus terrestris*. *The Psychological Record*, *14*, 383–387. [Runway; moss v empty goalbox]
- Robinson, J. S. (1953). Stimulus substitution and response learning in the earthworm. *Journal of Comparative and Physiological Psychology*, *46*, 262–266. [T-maze; careful analysis of locomotor path]
- Rosenkoetter, J. S., & Boice, R. (1975). Earthworm pheromones and T-maze performance. *Journal of Comparative and Physiological Psychology*, *88*, 904–910. [T-maze; pheromone release upon shock]
- Swartz, R. D. (1929). Modification of behavior in earthworms. *Journal of Comparative Psychology*, *9*, 17–33. [Y-maze; shock]
- Wyers, E. J., Smith, G. E., & Dinkes, I. (1974). Passive avoidance learning in the earthworm (*Lumbricus terrestris).* Journal of Comparative and Physiological Psychology, *86*, 157–163. [Runway; passive avoidance; saline as aversive stimulus]
- Yerkes, R. M. (1912). The intelligence of earthworms. *Journal of Animal Behavior*, *2*, 332–352. [T-maze; the first]
- Zellner, D. K. (1966) effects of removal and regeneration of the suprapharyngeal ganglion on learning, retention, extinction and negative movements in the earthworm *Lumbricus terrestris* L. *Physiology and Behavior*, *1*, 151–169. [T-maze; shock; dark goal box; surgery]

Pavlovian Learning

CLASSICAL CONDITIONING studies are a challenge in earthworms because of their limited sensory capabilities. Among the stimuli that have been employed are lights, vibrations, and odors. Responses examined include contractions, rearing, and withdrawal.

- Abramson, C. I., & Buckbee, D. A. (1995). Pseudoconditioning in earthworms (*Lumbricus terrestris*): Support for nonassociative explanations of classical conditioning phenomena through an olfactory paradigm. *Journal of Comparative Psychology*, *109*, 390–397. [CS(rose scent) — US(n-Butanol); CR: contraction]
- Bitterman, M. E. (1960). Toward a comparative psychology of learning. *American Psychologist*, *15*, 704–712. [CS — US(shock); attempt to record withdrawal mechanically]
- Bittner, L. H., Johnson, G. R., & Torrey, H. B. (1915). The earthworm and the method of trial. *Journal of Animal Behavior*, *5*, 61–65. [URs to light]
- Fields, T. A. (1970). Extended primary and higher order conditioning of earthworms. Masters thesis, Texas Tech. [CS(light) — US(shock); CR: contraction]
- Gardner, L. E. (1968). Retention and overhabituation of a dual-component response in *Lumbricus terrestris*. *Journal of Comparative and Physiological Psychology*, *66*, 315–318. [Habituation to vibratory stimulus]
- Gilpin, A. R., Ratner, S. C., & Glanville, B. B. (1978). Stimulus generalization of contraction response to light in earthworm. *Perceptual and Motor Skills*, *47*, 230. [Habituation to light stimuli; generalization]
- Herz, M. J., Peeke, H. V. S., & Wyers, E. J. (1967). Classical conditioning of the extension response in the earthworm. *Physiology and Behavior*, *2*, 409–411. [CS(light) — US(vibration)]
- Herz, M. J., Peeke, H. V. S., & Wyers, E. J. (1964). Temperature and conditioning in the earthworm *Lumbricus terrestris*. *Animal Behaviour*, *12*, 502–507. [CS(light) — US(vibration); better CRs at room temperature]
- Peeke, H. V. S., Herz, M. J., & Wyers, E. J. (1965). Amount of training, intermittent reinforcement and resistance to extinction of the conditioned withdrawal response in the earthworm (*Lumbricus terrestris*). *Animal Behaviour*, *13*, 566–570. [CS(vibration)—US(light); CR: rearing and withdrawal]

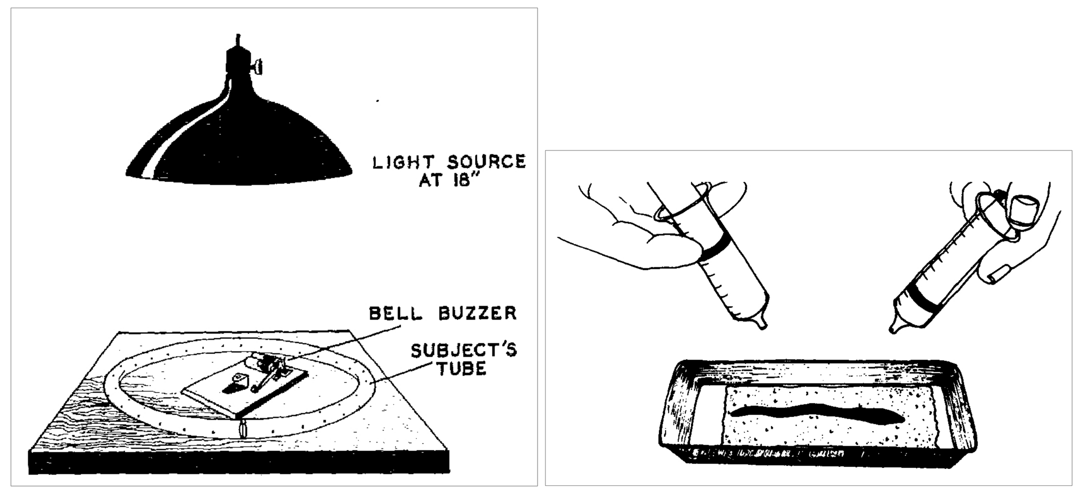

Figure 3: *Rattner & Miller's vibratory conditioning (left) and Abramson & Buckbee's olfactory conditioning (right)*

- Peeke, H. V. S., Herz, M. J., & Wyers, E. J. (1967). Forward conditioning, backward conditioning, and pseudoconditioning sensitization in the earthworm (*Lumbricus terrestris*). *Journal of Comparative and Physiological Psychology*, *64*, 534 – 536. [CS(vibration)—US(light); CR: rearing and withdrawal]
- Ratner, S. C., & Miller, K. (1959). Classical conditioning in earthworms, *Lumbricus terrestris*. *Journal of Comparative and Physiological Psychology*, *52*, 102–105. [CS(vibration) — US(light); CR: rearing and withdrawal]
- Ratner, S. C., & Miller, K. (1959). Effects of spacing of training and ganglion removal on conditioning in earthworms. *Journal of Comparative and Physiological Psychology*, *52*, 667–672. [CS(vibration) — US(light); CR: rearing and withdrawal]
- Ratner, S. C., & Stein,D. G. (1965). Responses of worms to light as a function of intertrial interval and ganglion removal. *Journal of Comparative and Physiological Psychology*, *59*, 301–305. [CS(vibration) — US(light); CR: rearing and withdrawal]
- Walton, W. R. (1927). Earthworms and light. *Science*, *66*, 132. [Insensitivity to red light]
- Watanabe, H., Takaya,T., Shimoi, T., Ogawa, H., Kitamura, Y., & Oka, K. (2005). Influence of mRNA and protein synthesis inhibitors on the long-term memory acquisition of classically conditioned earthworms. *Neurobiology of Learning and Memory*, *83*, 151 – 157. [CS(vibration) — US(light); CR: shrinking]
- Wyers, E. J., Peeke, H. V. S., & Herz, M. J. (1964). Partial reinforcement and resistance to extinction in the earthworm. *Journal of Comparative and Physiological Psychology*, *57*, 113–116. [CS(vibration) — US(light); CR: withdrawal; partial reinforcement]

Running Wheels

SEVERAL VERSIONS OF running wheels have been implemented for earthworms, with crawling speeds up to 30 cm/min reported. Wheels have been as simple as a piece of clear plastic tubing placed around the circumference of a tape reel, and as elaborate as a milled trackway in a machined piece of Plexiglas. In all cases, the mass of the wheel is minimized, and sensors detect rotational movement. My wheel is made from a red plastic flying disc. See CAMPUS.ALBION.EDU/WJWILSON/RESEARCH for a video of a worm in a wheel.

- Baldwin, F. M. (1917). Diurnal activity of the earthworm. *The Journal of Animal Behavior*, *7*, 187–190. [Vertical glass plates, not wheel; activity greatest at night]
- Burns, J. T., Scurti, P. J., & Furda, A. M. (2009). Darwin, earthworms & circadian rhythms: A fertile field for science fair experiments. *The American Biology Teacher*, *71*, 99 - 102. [Plastic tube around tape reel]
- Marian, R. W., & Abramson, C. I. (1982) . Earthworm behavior in a modified running wheel. *The Journal of Mind and Behavior*, *3*, 67–74. [Milled plastic; light/dark effects]
- McManus, F. E., Mendelson, T. & Wyers, E. J. (1982). The brain and central control in the earthworm. *Behavioral and neural Biology*, *35*, 1–16. [Milled plastic; supra- and subpharyngeal ganglia removal]
- McManus, F. E., & Wyers, E. J. (1978). A device for measuring patterns of locomotor behavior in the earthworm. *Behavior Research Methods & Instrumentation*, *10*, 398–400. [Milled plastic]

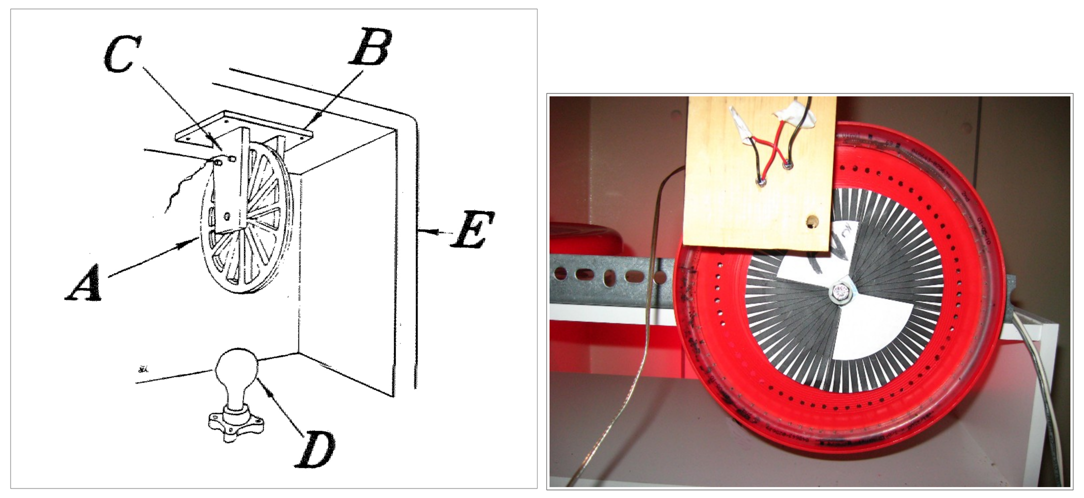

Figure 4: *Running wheels of Marion & Abramson (left) and the author (right)*

Surgery & Anatomical References

SOME SURGICAL MANIPULATIONS of the comparatively simple nervous systems of worms have tended to be crude. Ratner & Stein (1965) describe the removal of the anterior 5 segments:

> *The group. . . was not anesthetized; each S was placed on the dissecting board, the anterior five segments were cut off with a razor blade, and S was returned to its box for 48 hr.*

OTHERS, INCLUDING RATNER & STEIN in other surgeries described in the same paper, have suggested more refined procedures that involve small surgical incisions and careful removal of various ganglia. It is also possible to cut the fiber bundles connecting the supra- and subesophageal ganglia, or to divide the left and right sides of these ganglia.

- Bharucha-Reid, R. P. (1961) Neuroanatomical correlates of directional behavior in the earthworm. *Journal of Comparative and Physiological Psychology*, *54*, 337–339. [Supraesophageal ganglion removed; crossed control of movement]
- Blue, J. (1976). Effect of anterior ganglia removal on phototaxis in the earthworm (*Lumbricus terrestris*). *Bulletin of the Psychonomic Society*. *7*, 257–259. [Removed anterior 5 or 6 segments]
- Csoknya, M., Barna, J., & Elekes, K. (2002). Reorganization of the GABAergic system following brain extirpation in the earthworm (*Eisenia fetida*, annelida, oligchaeta). *Acta Biologica Hungarica*, *53*, 43–58. [GABAergic regrowth following brain removal]
- Hess. W. N. (1925), Nervous system of the earthworm *Lumbricus terrestris L.*. *The Journal of Morphology and Physiology*, *40*, 235–260. [Detailed neuroanatomy]
- Iwahara, S., & Fujita, O. (1965). Effect of intertrial interval and removal of the suprapharyngeal ganglion upon spontaneous alternation in the earthworm, *Pheretima communissima*. *Japanese Psychological Research*, *7*, 1–14. [Suprapharyngeal ganglion removed; decreased spontaneous alternation]

- Jiang, X. C., Inouchi, J., Wang, D., & Halpern, M. (1990). Purification and characterization of a chemoattractant from electric shock-induced earthworm secretion, its receptor binding, and signal transduction through the vomeronasal system of garter snakes. *The Journal of Biological Chemistry*, *265*, 8736–8733. [Describes coelemic secretion]
- Lore, A. B., Hubbell, J. A., Bobb Jr., D. S., Ballinger, M. L., Loftin, K. L., Smith, J. W., Smyers, M. E., Garcia, H. D. & Bittner, G. D. (1999). Rapid induction of functional and morphological continuity between severed ends of mammalian or earthworm myelinated axons. *The Journal of Neuroscience*, *19*, 2442–2454. ["Glue" restores function of severed giant axon]
- Prosser, C. L. (1934). The nervous system of the earthworm. *The Quarterly Review of Biology*, *9*, 181–200. [Detailed neuroanatomy]
- Ratner, S, C., & Gardner, L. E. (1968). Variables affecting responses of earthworms to light. *Journal of Comparative and Physiological Psychology*, *66*, 239–243. [Prostomium removal; response to light]
- Toman, J. E. P., & Sabelli, H. C. (1968). Neuropharmacology of earthworm giant fibers. *International Journal of Neuropharmacology*, *7*, 543–556.
- Ward, J.E., & Doolittle, J. H. (1973). The effect of anterior ganglia on forward movements in the earthworm. *Physiological Psychology*, *1*, 129–132. [Anterior 5 segments removed]

THE SEGMENTED NATURE of the earthworm offers the option for the ultimate within-subject control. Each segment appears to contain photoreceptors, touch sensors, chemoreceptors, and a pair of ganglia to process the incoming sensory information and control the segment's muscles. Transection of the nerve chain might result in two or more functional yet isolated segments capable of independent sensory and motor function. Perhaps different segments could be exposed simultaneously to presentations of CS and US in a paired or random manner, for a within-subject examination of Pavlovian conditioning. Different segments could be bathed in different doses of a drug for a within-subject dose-response curve determination. Interesting possibilities abound.

Acknowledgments

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