Technique Notes

Monitoring activity of *Drosophila* larvae: Impedance and video microscopy measures.

Cooper, Ann Simone, and Robin L. Cooper[1]. Department of Biology, University of Kentucky, Lexington, KY 40506-0225; Key Words: circadian, technique, insect, behavior; [1]To whom correspondence should be addressed; Phone: 859-257-5950; Fax: 859-257-1717; Email: RLCOOP1@pop.uky.edu

It is known that adult *Drosophila* show circadian patterns in movement and feeding behavior (Saunders, 1997). Circadian cycles in adults can be entrained by exposure to light as early as the embryo stage. In addition, entrainment for a circadian cycle can be induced in larval stages. The neurons responsible for this are controlled by the larval optic nerve (Bolwig's nerve) (Hassan *et al.*, 2000; Helfrich-Forster *et al.*, 2002; Malpel *et al.*, 2002). As far as we know there has yet to be a report on measures of circadian rhythms in *Drosophila* larvae, possibly because of the difficulty in such measurements.

To examine circadian rhythms in larval stages we used various approaches to image larvae from the 1st instar (1mm in length) to 3rd instar (4-5mm in length). Problems in monitoring larvae arise in that they burrow in their food. If the food is too deep one can not project white light through the food. In order to maintain the health of the animals over prolonged periods in examining patterns of movement, such as circadian rhythms, the animals must have access to food and be maintained in a humid environment. In addition, larval stages from the 1st to early 3rd instar are negative phototactic, while the late 3rd, usually referred to as the wandering 3rd, are positive phototactic. If food is present the larvae tend not to exhibit locomotion but to continually eat in a static location. Thus, the black mouth hooks and entire head are readily seen moving back and forth while the animals eat. The mouth hook movement is a common behavioral bioassay for *Drosophila* larvae (Neckameyer, 1996). If no food is provided the larvae will crawl over the surface thus making it hard to define its territory for monitoring. If Petri dishes are used the larvae crawl on the walls as well as on the lids. Thus, movements within different planes of focus make optical recording procedures difficult to use over an extended period of time. Additionally, the required humidity produces condensation on the surfaces of the containers. With uneven layers of food the small larvae are also readily lost visually for periods of time.

In this report, we present a technique termed the "Ant Farm Technique" of two glass plates (microscope slides; 75 x 25 mm; J. Melvin Freed Brand) narrowly spaced (1 to 1.5 mm) apart by a thin layer of larvae food (*e.g.* moist corn meal- a modified version of Lewis, 1960; Appendix) so that the larvae are able to be visualized within one plane of focus. To prevent larvae from crawling out from the edges of the two plates of the glass modeling clay is used. The larvae are very susceptible to dying if the carbon dioxide is not allowed to escape the chamber. The use of cotton on one edge is sufficient for gas exchange. Problems arise with a level platform since larvae become trapped in the moist food and the meniscus of the water and separate the chamber preventing exchange of gases with the outside. We have found that by slightly tilting the platform at 20 to 45 degrees the larvae remain, the majority of the time, with their head pointed downward and their tail containing the spiracles out above the food or within an air passage in the food layer.

This "Ant Farm Technique" also allows video imaging within a single plane with food of uniform thickness. Since in this configuration the larvae tend not to crawl rapidly throughout the food, but instead to eat and gradual move around in the 2D plane. Thus, the rapid head movements are readily observed and can be used as a desired behavioral trait to monitor for determining activity patterns. Larvae tend to eat continuously throughout their larval stages albeit at various rates. If monitoring the mouth hook movements is specifically desired then video microscopy will suffice with this arrangement. We have monitored instars from the 1st to the 3rd wander stage without disruption in this manner. White light was used 24 hours a day by use of a light box projecting light through the food. The light box used a 15 W neon light and was kept at a 30 cm distance from the glass plates. The microscope (adjustable zoom 0.67 to 4.5; World Precision Instrument; Model 501379) was tilted in order to remain parallel to the tilted glass plates. A 0.3× base objective and tube objective 0.5× were used to gain enough spatial resolution and magnification to cover a 1 cm by 2 cm rectangle. A mounted camera through a trinocular mount was used (Mintron, MTV; World Precision Instrument). The ambient temperature was maintained at 20°C with good circulation.

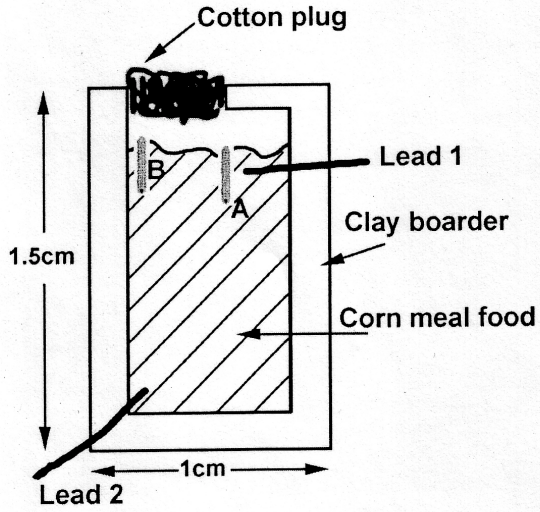

Figure 1. Schematic of the "Ant Farm" chamber. Two glass slides sandwich the clay border with the wires held firmly in place. The food is kept moist by the liquid tight borders with the exception of allowing gas exchange through the cotton plug at the top of the chamber.

Possibly this procedure could work when viewing in red light to examine if circadian behaviors existed in what the animal would perceive as a dark cycle. With our current video microscopy setup we did not have enough sensitivity to detect body wall or the mouth hook movements in red light. Thus, we developed an electrophysiological measure of dynamic resistance, also commonly referred to as an impedance measure, to determine any body movement from crawling to head movements for eating. Any slight change in resistance of the media induced by the animal's movement is able to be detected. With this recording arrangement, while the apparatus remains in total darkness, we were able to avoid even using red light. Studies are now underway to monitor circadian patterns with this technique (Cooper *et al.*, 2005).

Two insulated silver wires (diameter 0.005 inches and with the coating 0.008 inches; A-M systems, Inc., Carlsburg, WA) were placed through the modeling clay so the tips were exposed to the corn meal solution. These two wires need to span the chamber to insure a measure if the larvae moved within the chamber (Figure 1). The output of the impedance detectors (UFI, model 2991) was recorded on-line to a PowerMac 9500 via a MacLab/4s interface (ADInstruments). All events were measured and calibrated with the MacLab Chart software version 3.5.6 (ADInstruments, Australia) with an acquisition rate set at 400 points/sec. The deflections in the baseline can be calculated as activity over time. The impedance measures have been used in the past successfully to monitor heart rate in intact crayfish (Listerman *et al.*, 2000).

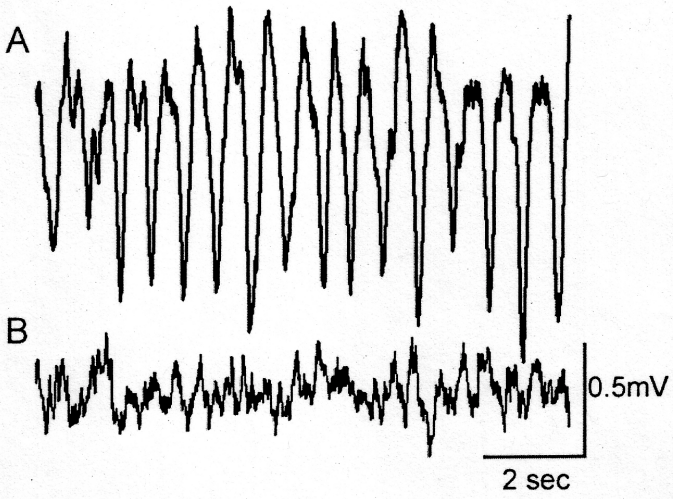

Figure 2. Impedance measures when the larva is close (A) to a lead (Position A in Figure 1) and far (B) from a lead (Position B in Figure 1). The entire trace is 10 seconds in duration.

Several larvae or single larva can be placed into a given chamber. However here we wanted to directly link movements of a larva with the impedance signals. As a larva approaches close to a lead, the deflection of the signal increases (Figure 2). To insure accurate measure of movements to impedance signals, carbon dioxide was used to anesthetize the larva while in the apparatus. When the larva ceased all movements the impedance measures flat lined. As the larva recovered and started to move and resume eating the impedance signals were also revived. This procedure can also be designed for many parallel units of single or multiple larvae for high throughput in screening *Drosophila* larvae in relation to mutants and drugs mixed with the food. A disadvantage in this technique is that the leads must remain in electrical contact through the media.

Appendix: *Drosophila* food: Water (17 l), agar (93 g), cornmeal (1,716 g), inactive yeast (310 g), sucrose (517 g), dextrose (1,033 g), phosphoric + propionic acid mix (164 ml distilled water to 836 ml of propionic acid. Add 917 ml distilled water to 83 ml of phosphoric acid. Combine the two diluted acid solutions to produce the acid mix -use 200 ml), and 1.6 vol tegosept in EtOH.

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References: Cooper, A.-S., H. Cooper, and R.L. Cooper 2005, Society for Integrative and Comparative Biology annual meeting. San Diego, CA, January 4-8; Hassan, J., M. Busto, B. Iyengar, and A.R. Campos 2000, Behav Genet. 30(1): 59-69; Helfrich-Forster, C, T. Edwards, K. Yasuyama, B. Wisotzki, S. Schneuwly, R. Stanewsky, I.A. Meinertzhagen, and A. Hofbauer 2002, J. Neurosci. 22(21): 9255-9266; Lewis, E.B., 1960, Dros. Inf. Serv. 34: 117-118; Listerman, L., J. Deskins, H. Bradacs, and R.L. Cooper 2000, Comp. Biochem. Physiol. A. 125: 251-264; Malpel, S., A. Klarsfeld, and F. Rouyer 2002, Develop. 129(6): 1443-1453; Neckameyer, W., 1996, Dev. Biol. 176: 209- 219; Saunders, D.S., 1997, Invert. Neurosci. 3(2-3): 155-164.

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Material contributed by
DROSOPHILA WORKERS

and arranged by
James N. Thompson, jr.
and
Jenna J. Hellack

prepared at
Department of Zoology
University of Oklahoma
Norman, Oklahoma 73019