Methods to measure circadian pattern in isolated adults.

Cooper, Ann S. Dept. of Biology, University of KY, Lexington, KY. 40506-0225 USA. Email: asc1029@hotmail.com.

Measuring circadian patterns in a pool of adults can have compounding variables to control, whereas isolated adults reduce social variables when addressing mechanisms driving a circadian pattern. A common approach to index circadian patterns is to monitor locomotor activity (Klarsfeld, 2003). However, monitoring single flies as compared to a population presents challenges. Here I report on two relatively inexpensive and simple approaches to index circadian patterns in single flies with readily accessible instrumentation.

After entrainment of a circadian pattern, one examines if the rhythm remains in the absence of the light cycle (Pittendrigh and Daan, 1976). This can be accomplished in total darkness or with continuous light. In order to detect activity of entrained adult flies a narrow glass tube was used. It was wide enough for the adult to turn around, but does not allow the adult to fly. The glass tube is Pyrex Disposable Pipette (serial number CGW 3597). I used a glass tube that is 5.1 cm in length for these studies.

At one end of the glass tube a clear rubber tube, ½ cm in length, was placed. This made it easy to remove and replace as needed. One end of the rubber tubing was for the foam stopper and the other end was for the standard *Drosophila* corn meal food (Figure 1). At the other end of the glass tube, foam was placed to allow ventilation.

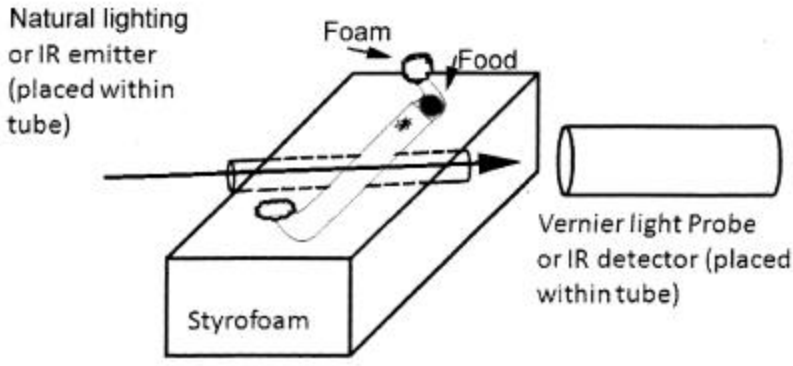


Figure 1.

For measuring the circadian pattern in continuous light I used a light probe (TI Light Probe, TILT-BTA) from Vernier (Vernier Software & Technology, Beaverton, OR, USA). The probe interfaces with a Vernier LabPro (commonly used in many high schools and introductory college science classes). The data are downloaded to a TI-83 hand calculator or directly stored on a computer. This probe is placed on a cut out of Styrofoam to hold it as well as the glass ally way tube (Figure 1). The light probe is placed directly against the glass tube and will detect each crossing of the light path. Normal room lighting may be used for the light source or any standard lighting within a temperature controlled incubator.

For measuring activity in constant darkness, an infrared (IR) light emitter (model 276-142, Radio Shack) was used in conjunction with the light probe (TI Light Probe, TILT-BTA) from Vernier as it is sensitive to IR.

An alternative approach to monitoring activity using different instrumentation was also examined for its feasibility. Activity in constant darkness or with a room well lit, the IR emitter can be used to monitor a fly when crossing an IR beam. The detector (model 276-142, Radio Shack) was

used instead of the Vernier light probe. The IR detector was connected to an impedance amplifier (UFI, model 2991, Morro Bay, CA, USA) in which the output is then passed through an AD board (MacLab 4s interface, ADInstruments, INC; Colorado Springs, CO, USA) before being collected on a computer with the MacLab Chart (version 5) software. As with the earlier method, Styrofoam is used to cut out holders for aligning the infrared light path and glass alley way tube. The emitter and detector were aligned by use of a larger plastic pipette that had a cut away for placing the glass fly tube (Figure 1).

With either approach, Vernier or impedance detector, the IR emitter (model 276-142, Radio Shack) can be powered by a 9V battery for about 24 hrs. I found the battery strength runs down quickly. Alternatively, use of a DC power source from a 120V AC to a 9V DC transformer is suggested to be used. Each emitter requires a 66 Ohm resistance on one lead. With the transformer as a power source a number of emitters can be used. I used 8 in parallel with 1 resistor in series from the transformer the 8 emitters in parallel. The rate of acquisition, not to over collect but to catch quick movements, is best about 25,000 samples/hour for the Vernier software. Collecting data for 4 hours or less in each set keeps files small enough as to readily open and analyze. The impedance amplifier with the MacLab 4s interface one can collect for 8 hours at 1 KHz to sufficiently detect movements and keep files small enough to manage.

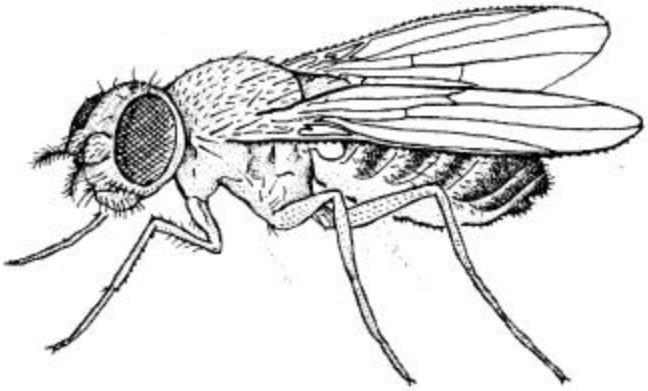
The responses from either method are then plotted as beam breaks over time for determining the activity of individual flies. These methods allow for various experimentations to be utilized, such as readily altering food sources, effects of compounds mixed with food and changes in environmental lighting (Sheward *et al*., 2007; Yoshii *et al*., 2007). Light conditions can be readily altered to total darkness or with visible light while still monitoring the adult locomotor activity if the IR emitter is used as a light source for the detector. Background level of absolute intensity on the Vernier detector will vary when using the IR emitter while the white light is turned on or off as the detectors pick up some of the white light signal.

I found the Vernier LabPro connected directly to a computer to be the easiest approach to set up and monitor activity. The impedance amplifier can saturate and requires monitoring often. Also the LabPro allows 4 probes to be connected simultaneously while the impedance amplifier monitors a signal detector. The net cost is also cheaper with the Vernier hardware as 4 detectors and one Vernier LabPro costs about $500 (USD) while each impedance amplifier costs about $350 (USD).

Acknowledgments: I thank Dr. R.L. Cooper at the University of KY for his advice and help with this study.

References: Klarsfeld, A., J.C. Leloup, and F. Rouyer 2003, Behavioural Processes 64: 161-175; Pittendrigh, C.S., and S. Daan 1976, J. Comp. Physiol. [A] 106: 223–252; Sheward, W.J., E.S. Maywood, K.L. French, J.M. Horn, M.H. Hastings, J.R. Seckl, M.C. Holmes, and A.J. Harmar 2007, J. Neurosci. 27(16): 4351-4358; Yoshii, T., K. Fujii, and K. Tomioka 2007, J. Biol. Rhythms 22(2): 103-114.

Drosophila Information Service



Number
90

December 2007

Prepared at the
Department of Zoology
University of Oklahoma
Norman, OK 73019 U.S.A.