Technique Notes

The free-running period of *Drosophila melanogaster* is not affected by the length of the tube in a *Drosophila* Activity Monitor (DAM).

Seggio, Joseph A. Department of Biology, Bridgewater State University, 24 Park Avenue, Bridgewater, MA 02324; jseggio@bridgew.edu

Drosophila melanogaster has been a widely used model to study the circadian rhythm since the creation of the *period* mutants (Konopka and Benzer, 1971), as there are many similarities in circadian function to mammals at both the molecular and behavioral levels (Helfrich-Foster, 2004). Many different devices were invented and used to observe the behavioral circadian rhythm in fruit flies, but the most commonly used are the *Drosophila* Activity Monitors (DAMs), made by the company Trikinetics Inc. (Waltham, MA) (Klarsfeld *et al.*, 2003). Several articles and chapters describe the procedures for using activity monitors and provide detailed instructions from setting-up the monitors, computers, software, and wiring (Klarsfeld *et al.*, 2003; Rosato and Kyriacou, 2006; Zordan *et al.*, 2007; Chiu *et al.*, 2010; Pfeiffenberger *et al.*, 2010). Basically, the DAMs monitor the activity of individual flies housed in a tube by counting the number of infrared beam crossings within a specific time-frame or bin (which can range from 1 sec to 60 min), and subsequently compiling the activity in a raw data text file. It is then necessary to utilize a separate data-analysis program, such as ClockLab (Actimetrics, Wilmette, IL) or Actiview (Minimitter – Philips Respironics, Bend, OR) in order to extract the information from the raw-data file and to produce an actogram, which is a graphical representation of the individual fly's activity rhythm. The use of fruit flies and DAMs to study the circadian clock is a powerful, yet cost-effective, method to use in both a research and classroom setting (Seggio, 2011).

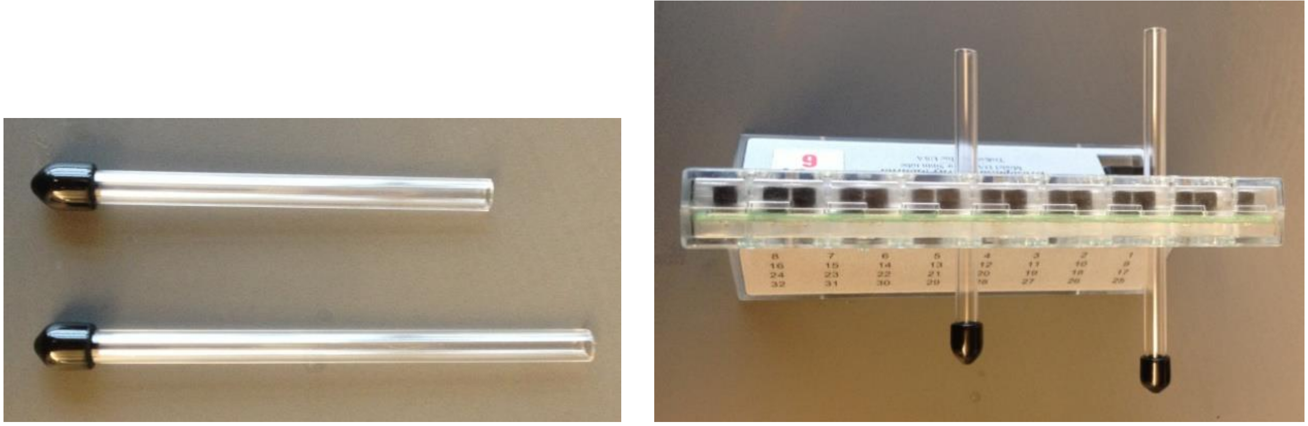

Figure 1. *Left:* A 5 × 65 mm polycarbonate tube with black cap (top) compared with a 5 × 80 mm tube (bottom). *Right:* When placed into a DAM, the 80 mm tube (right) has more room for motion compared to the 65 mm tube (left).

The majority of the projects published using the DAMs have used a 5 × 65 mm tube to house the individual fly, including Seggio *et al.* (2012) and Oh *et al.* (2013), as well as at least thirty other articles since 2012. Recently, Trikinetics has offered a larger tube size, 5 × 80 mm, for use in the DAMs, but few researchers have used it to date. Lone and Sharma (2012) have recently published using the longer tube, according to a recent google.scholar search for "Trikinetics" and "80 mm"/"80mm" (although Rosato and Kyriacou, 2006, and Zordan *et al.*, 2007, make note of that option in their reviews). This project aims to uncover any differences in circadian activity levels and free-running period between the 65 mm and 80 mm tubes.

Methods

Canton-S (*CS*), *Oregon-R* (*Or-R*), *period Short* (*perS*), *period Long* (*perL*) *Drosophila melanogaster*, reared at 25ºC in a 12:12 Light:Dark (LD) photoperiod were used throughout this study. Flies were raised on instant *Drosophila* medium Formula 4-24, blue, (Carolina Biological Supply Company, Burlington, NC) with 5 mL food and 11 mL of water per vial. Randomly selected one-day-eclosed adults were individually monitored for locomotor activity and circadian free-running period. Single adult flies were placed into individual polycarbonate tubes (either 5 × 65 or 5 × 80 mm tubes) containing approximately 10 mm of 5% (w/v) sucrose and 2% agar (w/v) food with a plastic cap on one end and a cotton plug approximately 10 mm in length on the other end, and placed into activity monitors (DAM2 – Trikinetics Inc., Waltham, MA). The DAMs were placed into an incubator with approximately 200 lux fluorescent light in a 12:12 LD cycle at 25ºC for 3 full days. Following the 3 days in 12:12 LD treatment, measurements continued without interruption for 7-10 additional days in constant darkness (DD) to assay the free-running circadian period of locomotor activity. Activity was monitored using the Trikinetics DAM3 data collection software with activity counts collected in 10-min bins, and assembled into data files for individual flies using the DAMFileScan Software. Mean activity levels and circadian period (an average of the chi-square periodogram and visual inspection) in DD for individual flies were analyzed using ClockLab (Actimetrics, Wilmette, IL). Two-way ANOVAs were used to detect significant differences in mean activity and period between tube-length, fly type, and tube by genotype interaction for the wild-type and *period* mutant flies.

Results

Table 1 shows the mean activity in LD and DD, as well as the free-running period for each of the four genotypes. *CS* and *Or-R* were shown to have significantly different periods ($F_{1,147} = 56.4$, $p < 0.001$), with *CS* having a longer rhythm; however, *Or-R* was found to have higher activity amplitude than *CS* in both LD ($p < 0.001$) and DD ($p < 0.001$). There were no tube-length by genotype interactions for LD, DD, or free-running period (all $p > 0.10$); *i.e.*, Canton-S in 65 mm tubes did not differ from *CS* in 80 mm tubes (the same being observed for *Or-R*). Obviously, *perL* and *perS* have significantly different periods ($F_{1,105} = 4621$, $p < 0.001$). *perS* was found to have much higher activity amplitude in both LD ($p < 0.001$) and DD ($p = 0.001$) than *perL*, a result that was previously found by Ahmad *et al.*, 2013. As before, there was no significant difference found for either tube-length or tube-length by genotype interaction (all $p > 0.10$) for LD and DD activity or free-running period for the *period* mutants.

Based on these results, it appears that there are no differences between the 65 mm and 80 mm tubes in LD or DD activity in terms of beam crosses per 10 min bin or the free-running period among two widely-used wild-type flies and two *period* mutants. While average beam crosses per 10 min bin were not different among any of the four interactions, it is not known whether the larger tube will alter other activity parameters, such as average bout length, counts per bout, bouts per day, or Light:Dark activity ratio.

Table 1. Average activity (number of beam crossings per 10 min bin ± SEM) in LD and DD, as well as free-running period (hours ±SEM) for the two wild-type flies (CS and Or-R) and the period mutants (perS and perL).

Genotype	Tube Length	N (Sample)	Activity in LD	Activity in DD	Period
Canton-S	65mm	35	5.00 ± .31	5.13 ± .31	24.34 ± .03
(*CS*)	80mm	25	4.28 ± .30	5.14 ± .35	24.33 ± .02
Oregon-R	65mm	45	6.35 ± .37	6.56 ± .31	24.05 ± .04
(*Or-R*)	80mm	46	5.92 ± .44	6.47 ± .48	24.04 ± .04
period Short	65mm	32	6.87 ± .60	7.25 ± .57	19.26 ± .12
(*perS*)	80mm	23	6.79 ± .58	7.65 ± .81	19.29 ± .10
period Long	65mm	28	4.82 ± .37	4.77 ± .42	28.37 ± .11
(*perL*)	80mm	26	5.29 ± .52	4.00 ± .39	28.44 ± .18

References: Ahmad, S.T., S.B. Steinmetz, H.M. Bussey, B. Possidente, and J.A. Seggio 2013, Behav. Brain Res. 241: 50-55; Chiu, J.C., K.H. Low, D.H. Pike, E. Yildirim, and I. Edery 2010, J. Vis. Exp. (43); Helfrich-Forster, C., 2004, J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. 190(8): 601-613; Klarsfeld, A., J.C. Leloup, and F. Rouyer 2003, Behav. Processes 64(2): 161-175; Konopka, R.J., and S. Benzer 1971, Proc. Natl. Acad. Sci. USA 68(9): 2112-2116; Lone, S.R., and V.K. Sharma 2012, J. Biol. Rhythms 27(2): 107-116; Oh, Y., D. Jang, J.Y. Sonn, and J. Choe 2013, PLoS ONE 8(7): e68269; Pfeiffenberger, C., B.C. Lear, K.P. Keegan, and R. Allada 2010, Cold Spring Harb. Protoc. 2010(11): pdb prot5518; Rosato, E., and C.P. Kyriacou 2006, Nat. Protoc. 1(2): 559-568; Seggio, J.A., 2011, Dros. Inf. Serv. 94: 170-173; Seggio, J.A., B. Possidente, and S.T. Ahmad 2012, Chronobiol. Int. 29(1): 75-81; Zordan, M.A., C. Benna, and G. Mazzotta 2007, Methods Mol. Biol. 362: 67-81.

An efficient, practical, and reliable *Drosophila* trap.

Freda, P.J.[1], and J.M. Braverman[1*]. [1]Department of Biology, Saint Joseph's University, Philadelphia, PA, USA; *corresponding author (E-mail: jbraverm@sju.edu).

A good *Drosophila* trap should be made of materials that are inexpensive and readily available. Also, the materials should be sturdy enough to be used outdoors. Additionally, a trap should be simple enough that anyone can assemble it quickly. The trap of Medeiros and Klaczko (1999) is well designed, but improvements and simplifications are possible. Using their work as a foundation, an efficient, practical, and reliable trap for live *Drosophila* specimen collection was designed.