Utilizing *Drosophila* Activity Monitors (DAMs) in an undergraduate teaching and research setting.

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The fruit fly model system is suitable for efficient genetic, neural, and behavioral analysis (Mackay and Anholt, 2006), including analyzing the circadian rhythm. *Drosophila melanogaster* has been a widely used model for studying circadian mutants since the isolation of the *period* (per) mutants (Konopka and Benzer, 1971). Further explorations have also concluded that there exists a high degree of homology between the molecular circadian clock mechanism of *Drosophila* and mammals (Helfrich-Foster, 2004), which makes *Drosophila* an excellent model for examining circadian clock function.

As Klarsfeld *et al.* (2003) reports, studies have shown that different devices were invented to observe the behavioral circadian locomotor activity rhythm in fruit flies (Hamblen *et al.*, 1986; Helfrich 1986), but currently, the most widely used are the *Drosophila* Activity Monitors (DAMs), such as the devices built by the company Trikinetics (Waltham, MA) (Klarsfeld *et al.*, 2003) (Figure 1a). Previous articles describe the actual process of setting-up the computers and monitors, and placing the flies into the individual monitor tubes (Rosato and Kyriacou, 2006) (Figure 1b) – in particular, Chiu *et al.* (2010) provides detailed instructions beginning with incubator set-up to data analyses. Essentially, the DAMs monitor the activity of individual flies by counting the number of infrared beam crossings within a 10-min time period or bin, and subsequently compiling the activity in a raw data text file (Klarsfeld *et al.*, 2003; Rosato and Kyriacou, 2006; Chiu *et al.*, 2010; Pfeiffenberger *et al.*, 2010). It is then necessary to utilize a separate data-analysis program, such as ClockLab (Actimetrics, Wilmette, IL) or VitalView (Minimitter, 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.

Figure 1a (left) and 1b (right). a) An example of a DAM, specifically model DAM2. The DAM2 connects to the computer and power source via the standard 4-wire telephone jack in the bottom left corner. b) Thirty-two individual flies are placed into tubes with an agar-sucrose food, covered with a black cap (shown), and capped off on the other end with cotton (not shown).

Monitoring the locomotor activity in individual flies can uncover altered behavior, including the free-running rhythm, over the course of several days or even weeks (Pfeiffenberger *et al*., 2010). Observing individual flies allows the investigator or student to easily discern rhythmic from arrhythmic, and mutant from non-mutant individuals, through quick examination of the actogram (Figure 2). This is in contrast to eclosion experiments in which the population will be interpreted as arrhythmic if constituted of rhythmic, albeit unsynchronized individuals (Konopka and Benzer, 1971; Klarsfeld *et al*., 2003). Indeed, recording the locomotor activity of an individual fly was, and still is, an extremely powerful tool that helps identify circadian rhythm mutants (Klarsfeld *et al*., 2003; Zordan *et al*., 2007; Pfeiffenberger *et al*., 2010). Therefore, I submit that the DAMs, similar to the monitors from Trikinetics, are an excellent, although unfortunately underutilized, tool in teaching undergraduate students concepts of the circadian clock, behavior, genetics, and neuroscience.

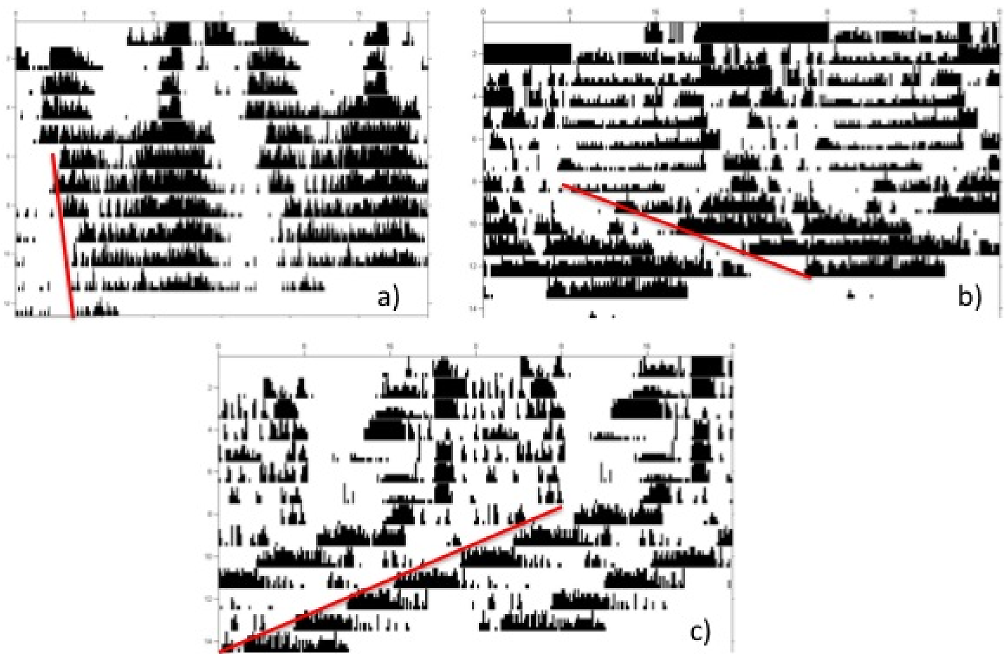

Figure 2. Three representative, double-plotted actograms showing the free-running periods from wild-type and *period* mutant *D. melanogaster*. All three actograms were generated using the program ClockLab. The red line indicates the slope of the free-running period. a) Canton-S wild-type, b) per^{L}, c) per^{S}. The wild-type fly has a period approximately 24.5 h, while the long and short mutants (per^{L} and per^{S}) have average periods of 28.6 h and 19.5 h, respectively. The per^{L} and per^{S} are the mutant flies from the Konopka and Benzer (1971) study and were provided generously by Dr. Michael Rosbash.

The teaching and research projects conducted in an undergraduate setting should fulfill the following criteria: 1) be in accordance with the curriculum and reinforce the theoretical concepts learned in the classroom, 2) train students to conduct a series of experiments that achieve a set of objectives in a timely manner, 3) prepare them for future scientific careers as graduate or medical students and beyond, and 4) be feasible in terms of available funds. The DAMs fulfill all of these conditions as described through the following.

1) Research with undergraduates should reinforce the concepts learned in their upper-level elective courses (*i.e.*, 300- or 400-level), regardless of their sub-discipline in biology. As both a

neuroscience instructor and a circadian rhythm researcher at a primarily undergraduate institution, I rely heavily on the use of DAMs as an important teaching tool in both the classroom and research setting. In both my Neurobiology and Biological Clocks classes, students design group research projects with only directional input from me. These projects investigate simple yet informative questions posed by the students and serve as the final project in each of my two upper-level electives. I highly encourage the students to use the DAMs in their work, because in doing so they can easily observe a significant and quantitative behavior in the flies. I have observed that when given the opportunity, my undergraduate students develop extremely creative and original projects that utilize: a) easily accessible pharmacological agents (*i.e.*, over-the-counter solutions, supermarket items, chemicals the department already possesses, and so forth), b) the plethora of *Drosophila* mutants available from Bloomington Stocks (Bloomington, IN) and c) the DAMs, for their laboratory-class assignments.

2) The students can design experiments that may reveal the activity behavior of certain genetic backgrounds or even crosses of their own design, including some potentially unusual ones. Furthermore, they can test the effects of environmental or pharmacological agents on the following circadian parameters: a) free-running period, b) the capacity to adapt to day-night cycles with photoperiods of differing length (or perhaps use a skeleton photoperiod), and/or c) the phase of the circadian activity cycles with respect to the stimulus (Zordan *et al.*, 2007). In addition to the activity monitors, Trikinetics also sells devices that can examine other aspects of the fly's behavior by which students can further explore their interests. Examples of projects students may investigate can include showing how different pharmacological agents can alter the fly's ability to maintain balance on a rotarod, or how sleep deprivation affects the activity of the fly. Students can subsequently create projects that utilize multiple tools and determine if different agents or genetic factors affect multiple behaviors. The DAMs also have the advantage of collecting relatively large amounts of data in a very small amount of time; the students can gather results for their in-class projects in about two weeks. In a class-laboratory setting, the instructor can illustrate how to use the DAMs during the first lab week, and then have the students complete different lab assignments during the second week while the activity is being monitored. During the third lab week, the instructor can focus on data analysis instruction. Since the DAMs and the other tools are simple and efficient to use, the students can focus on designing creative experiments, rather than concentrating primarily on the technical aspects of the study.

3) Since the DAMs allow for continuous recordings of several flies (32 per activity monitor), they can be used as a "high-throughput" way to study circadian rhythm and locomotor activity (Pfeiffenberger *et al.*, 2010). In fact, many journal articles have been published from prestigious research institutions that utilize the DAMs in their research. These studies include recent publications from both sleep (Parisky *et al.*, 2008; Wu *et al.*, 2008; Harbison *et al.*, 2009; Weber *et al.*, 2009) and circadian laboratories (Zhang *et al.*, 2009; Beaver *et al.*, 2010). A major advantage of circadian rhythm research is the minimal physical maintenance required after the flies are placed into the monitors. The undergraduate students are not relegated to performing only "grunt-work", such as cleaning equipment or waste, preparing solutions, labeling and preparing petri dishes, and so forth. They are afforded the opportunity to participate in authentic scientific inquiry, by designing the project, maintaining the fly stocks, which can include crossing different genetic strains, and anesthetizing the flies which are the essential technical skills required for participation in any *Drosophila* laboratory at the graduate level. Not only can the students create the protocols for the experiment, they can also play an important role in the data analyses and form conclusions based on their results. After the initial setup, a computer program collects the circadian activity data, offering the students the opportunity take part in calculating the circadian parameters themselves and running statistical analyses on the results, which ultimately could lead to student publication.

4) The DAMs from Trikinetics are also extremely cost effective. Four activity monitors, 200 re-usable monitor tubes and caps, as well as all of the wiring accessories (which have the capability to record the activity from 128 flies) can be purchased from Trikinetics for approximately $2,500. The only additional required equipment would be a *Drosophila* incubator, which all fly-laboratories would have. In addition, Trikinetics provides all of the computer software for recording the activity of the individual flies free of charge. Also needed is a computer program that can analyze the text files. Some simple programs that can produce an actogram or calculate the free-running period can be downloaded from the Internet at no cost (http://www.circadian.org/softwar.html), or more advanced software can be purchased from either Actimetrics or Minimitter. Once the initial system is assembled, instructors can utilize this paradigm year-after-year in the lab-section coursework of a Neuroscience, Biological Clocks, Animal Behavior, or Genetics class for no more than the cost of maintaining the fly stocks.

In using the DAMs, simple experiments can be conducted providing an excellent medium to teach undergraduates a scientifically sound method that is currently used in the fields of genetics and neuroscience. The students can address a number of questions in which they are interested, and these devices can allow the students a tremendous amount of creativity and flexibility in designing the experiments for their courses or in collaboration with their research advisor. These reasons make the *Drosophila* Activity Monitors excellent models and tools for examining activity behavior and circadian clock function in a classroom or small research setting.

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