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Automating behavior analysis in *Drosophila melanogaster* in a large undergraduate neuroscience laboratory course

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Abstract

NE203 Principles in Neuroscience is a large (150-student) required laboratory course. Because of its large student enrollment and new inquiry-based approach, the course recently went through a major overhaul in 2019. Curriculum switched from performing surgery on rats to using fruit flies. We ran a pilot term in the fall of 2021 and we now present our progress following the incorporation and optimization from the fall terms of 2022 and 2023. Students use *Drosophila melanogaster* as an experimental animal to ask original questions in systems neuroscience. In the first month, students are introduced to the field of neurogenetics and become familiar with common genetic tools used in fruit flies. The second month, students work on proposing an independent group project by collecting their own pilot data and writing an NIH-style grant proposal. This proposal is "peer-reviewed" and students are allowed to modify their experimental design and approach with feedback from the instructional team. In the final month, students continue collecting data and formally present their final grant proposal. Thus far, the introduction of the activity monitoring system has more than doubled student interest in utilizing automated behavioral monitoring in their proposals. Supported by the ABLE Roberta Williams Laboratory Teaching Initiative Grant.

Keywords: cell biology, genetics, behavioral assay, inquiry-based learning

Link to Supplemental Materials: https://doi.org/10.37590/able.v44.sup7

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INTRODUCTION

The goal of this exercise is to facilitate student collection of experimental behavioral data. A common behavioral screen that most student projects would have benefited from last semester involves activity monitoring. This instrument can automatically screen many fly lines at once and generate activity data that can be used for analyzing motor behavior and even more sophisticated sleep/wake patterns of circadian rhythmicity and learning/memory place conditioning assays. These results could be used for grant proposals, fellowships, publications, and/or professional presentations by the students.

This activity is part of a sophomore-level neuroscience course in which students use *Drosophila melanogaster* as a lens through which to study core neuroscience principles such as behavior analysis and neuroanatomy. In the latter half of the semester, students work on an independent, group research project in which they employ the gal4/UAS system to manipulate and fluorescently label known neurons in programming behavior(s) of their choice. As a program, we have built a repository of fly lines that students can mix or match to control many different types of cells to manipulate function. For example, with the powerful genetic tools offered in fruit fly genetics, students can use light, toxins, ion channels, and/or temperature to activate or inactivate select cells in awake-living flies across developmental stages (larval, pupal, adult) to investigate brain:behavior relationships. Students begin their independent projects by first learning how to perform genetic crosses, sex/handle flies, grow/amplify cell cultures, dissect and visualize Drosophila neuroanatomy, and quantify commonly studied Drosophila behaviors. After surveying primary literature on a specific neurobiological mechanism underlying a behavior of interest that is not well understood in the field of neuroscience, students search for available fly lines either commercially or privately via the Bloomington Stock Center or by reaching out to individual research labs.

Next, groups begin designing and building a behavioral assay to experimentally screen their flies. It is at this phase of the independent project that an activity monitor would add significant value in data collection and analysis. Because most students have not worked on this scale, performing behavioral tests in tiny organisms like fruit flies poses unique technical challenges for almost every group. One way to streamline behavioral data acquisition for student groups is through activity monitoring. Individual flies are aspirated into small cylindrical tubes retrofitted with sensors that detect beam breaks of light when flies move back and forth in hollow glass or plastic vials. Total number of beam breaks over time and space are calculated for each fly. This data can then be processed and visualized in several ways to measure total activity, sleep/wake cycles, and even adapted for assessments of place conditioning – an assay of learning and memory.

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STUDENT OUTLINE

Objectives

Drosophila Activity Monitor (DAM) setup training

Isolate Male Drosophila Melanogaster into individual vials

Deconvolute movement data collection for 48-72 hours

Plot deconvoluted data

Approach and Rationale

In lab this semester, you have been using the gal4/UAS system to manipulate and label neurons then study behaviors of Drosophila. One observable behavior is overall activity: how much flies move over a 24-hour period.

Today you will learn how to set up a Drosophila activity monitoring assay, how to collect the data, and how to extract information from the raw data. This procedure can be used to measure a variety of behaviors: sleep/wake cycles, total activity, place conditioning, and more.

Methods and Data Collection

Activity 1: Learn to process the DAM output using DAMFileScan113X software with sample data.

Activity 2: Capture and isolate male flies into assay vials

Activity 3: Outline, in detail, your plan for a research proposal in your Electronic Lab Notebook (ELN)

Postlab: Download the collected data from your section's files from the class LMS page. Process the data as in Activity 1, and using the Excel Template on Blackboard, plot your data as described in the Postlab Assignment Sheet. Please see your Lab Manual Handout for detailed instructions on data analysis, fly handling, and planning out your research project.

Data Analysis

The data processing is the most significant part of this module. Activity 2 in your Handout is designed to show you, step-by-step, how to create a usable output that can be plotted in Excel. After the course of the experiment has finished (48+ hours), your instructors will download the collected database files, and make them available on the LMS. You will then take these files and process them using the free DAMFileScan113X software. Be sure to follow the instructions in the Handout-especially the part that describes the identity of the columns.

When plotting, try to think about a practical way to have the data binned (how many hours to have the software sum the movements and combine that count). A great place to start is to bin them every 24 hours, as this will generate output in your file that will show the overall rhythms. From there, you can identify/align light/dark cycles, and then set your x-axis to be time of day after binning for every 12 hours. Much shorter periodicities can be done, but they tend to show a significant amount of noise.

Discussion

The main goal of this week's exercise is to improve your ability to design experiments and enhance your understanding of behavioral assays that can be used to evaluate experimental outcomes. This week, you are tasked with setting up a behavioral monitoring assay using *Drosophila melanogaster* as well as analyzing and exploring the outcomes of the assay. With the knowledge and skills you gain from this week's lab, you should be able to design your own behavioral assay to use this semester to evaluate your group's novel research question. This may include general activity analysis, or it may involve designing an experiment that examines place preference or sleep-wake

cycles. For example, you may want to design an experiment that evaluates whether optogenetic inactivation of dopaminergic neurons in Drosophila alters their preference for standard vs. sucrose-enhanced food options. Another student group may be interested in activating serotonergic neurons with temperature and evaluating how this affects Drosophila place preference when presented with an aversive vs. appetitive scent. Over the course of the rest of the semester, you will be equipped with the skillset to design a behavioral assay and analyze the results to collect meaningful data that will provide insight into your research question.

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MATERIALS

Equipment needed:

- 25 (or one per pair of students) stereo dissecting microscopes (above specimen-lit, 7x-45x)
- Fume hood in lab (helpful, but not imperative)
- 25 fine paintbrushes for fly manipulation
- 25 3-inch x 5-inch index cards
- 32 DAM vials and caps (Trikinetics, USA)
    - 32 x PGT5x65 Pyrex Glass tubes or 32 x PPT5x65 Polycarbonate tubes
    - 32 x CAP5-Black caps
    - A small amount of food should be placed at one end of the vial and covered with a cap
- Drosophila Activity Monitor (DAM) unit (Trikinetics, USA)
    - DAM5H-4 or DAM5H
- PSIU9 (Power Supply Interface Unit) (Trikinetics, USA)
- Monitor cable (included with DAM unit)

Supplies needed:

- FlyNap (Carolina, USA)
- Nitrile globes (S, M, L)
- Small 100-250 mL glass beakers
- Kimwipes
- 70% ethanol for cleanup
- Cotton balls

One Mac or PC with the DAMSystem3 software and an available USB port is required for data collection. Students should download the DAMFileScan113X software on their personal computers. Software is available for download at https://trikinetics.com/. Trikinetics price list and purchase instructions can be found at https://trikinetics.com/Downloads/DAMSystem%20Price%20List%202023.2.pdf.

NOTES FOR THE INSTRUCTOR

This laboratory course aims to equip students with proficiency in designing experiments and employing evidence-based reasoning through the completion of inquiry-based research projects in neuroscience. This curriculum provides students with early exposure to diverse model systems, technologies, and data science methods, facilitating a comprehensive foundation.

The NE 203 laboratory course revolves around independent research projects utilizing electrophysiological techniques and genetic tools to map neural behavior representations in invertebrates. Over the course of the semester, students will actively engage with the scientific method by critically assessing existing literature, identifying gaps in theoretical knowledge, formulating testable hypotheses, and subsequently executing experiments. Moreover, students will delve into the analysis of experimental data, substantiate claims based on evidence, and effectively communicate the broader theoretical implications of their findings. This communication will occur through a formal group presentation, which will be recorded, as well as the composition of a grant proposal. Throughout this process, students will refine their scientific communication skills and foster the ability to collaborate harmoniously within a scientific community while functioning as a cohesive team.

The NE 203 Labs demand that students acquire theoretical and technical knowledge that extends beyond the content covered explicitly in the NE 203 lecture. However, all lab projects have been meticulously designed to reinforce concepts taught in the lecture and to offer exposure to state-of-the-art techniques employed in contemporary neuroscience research. The experiments are structured to provide students with genuine, immersive experiences mirroring those undertaken in neuroscience research labs across the globe. By the conclusion of this course, our aspiration is that students will possess insight into the methodologies which neuroscientists employ to

prepare for and execute authentic experiments. Moreover, students will have gained an appreciation for how intricate functions of the nervous system can be discerned through meticulously designed studies.

The benefits of Course-based Undergraduate Research (CURE) are numerous and well documented. For students at Primarily Undergraduate Institutions (PUI), these provide high-impact research experiences that can culminate in retention in STEM careers and motivation to pursue graduate level education. They provide opportunities for students to make discoveries, collaborate, engage in meaningful research and develop a sense of ownership of their lab work. For faculty, especially at PUI, these provide tractable models of modern, collaborative science and move toward the complex, interdisciplinary nature of scientific investigation as an effective platform for integrating the goals of research and education. A wide variety of successful CUREs have been developed with different research themes, however only a handful of CUREs currently prioritize on the benefits of collaborative research across institutions. Two collaborative CUREs that are widely reported and highly successful are the malate dehydrogenase CURE (Bell et al. 2020) and the HHMI SEA- PHAGES CURE (Staub et al. 2016). Our CURE, Experiential Collaborative Parasite Research across institutions (ECoPaR) provided students an opportunity to engage in a cross-institutional, cross- disciplinary research experience and effectively contribute to ongoing Kinetoplastid research. This collaboration was between students and faculty of University of Mary Washington, Georgia State University and Albright University.

Our student body encompasses a diverse array of disciplines and academic backgrounds, spanning fields from computer science to philosophy and engineering. During the pilot semester of our laboratory course, we witnessed an impressive display of innovation and creativity among our students. Many of the projects undertaken during this period demonstrated both scientific rigor and feasibility. The novelty of these projects was significant enough to warrant further and more extensive exploration, supported by guided supervision.

Although the inquiry-based nature of independent projects might lead some groups to opt against employing an activity monitor as a central component of their research, it is advisable for all students to consider integrating activity monitoring for movement as a crucial positive/negative control. This analytical approach will empower groups to discern any unintended or off-target effects stemming from their neuronal manipulations. For instance, depending on the specific neurobiological systems being manipulated, flies might exhibit altered movement or sleep patterns, potentially confounding students' data interpretation. The acquisition of this type of data would not only enhance the value of students' grant proposals but also furnish a valuable educational opportunity for mastering statistical analysis and the visualization of behavioral data. The introduction of more rigorous analytical techniques for behavioral analysis stood as a fundamental objective in implementing the DAM systems.

To reiterate, the proposed laboratory module constitutes an initial training stage, equipping students with essential techniques. Subsequently, they will leverage this foundational knowledge to devise genetically-controlled fly crosses, innovate novel behavioral assays, and ultimately formulate a comprehensive research proposal backed by preliminary data. With meticulous guidance on the utilization of these devices, we have witnessed a notable upsurge in students' interest in incorporating them within their proposals. In partnership with Boston University's Writing Program, we enlist writing consultants to offer personalized training for proposal development. This course marks the second instance where students fulfill the mandatory HUB core credits for "Writing Intensive." These consultants, who are senior undergraduates familiar with the course and currently enrolled in STEM writing instruction at the Wheelock School of Education, provide valuable training. Their involvement is in addition to our team of Learning Assistants who deliver prelab talks, conduct training workshops, and provide hands-on support alongside students during the laboratory sessions.

Online Resources

https://trikinetics.com
https://trikinetics.com/Downloads/DAMSystem%20Price%20List%202023.2.pdf
https://trikinetics.com/Downloads/DAM5H%20Data%20Sheet.pdf
https://trikinetics.com/Downloads/DAMSystem%20Overview%202108.pdf
https://trikinetics.com/Downloads/DAMSystem3%20Software%20Data%20Sheet.pdf

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CITED REFERENCES

Brinson MM. 2022. Developing a novel place preference assay to compare drosophila species over time. Honors Theses. 2581. https://egrove.olemiss.edu/hon_thesis/2581

Chiu JC, Low KH, Pike DH, Yildirim E, Edery I. 2010. Assaying locomotor activity to study circadian rhythms and sleep parameters in Drosophila. Journal of Visualized Experiments. 28(43): 2157.

McParland AL, Follansbee TL, Ganter GK. 2014. Measurements of larval activity in the Drosophila Activity Monitor. Journal of Visualized Experiments. (98):52684.

Sun J, Xu AQ, Giraud J, Poppinga H, Riemensperger T, Fiala A, Birman S. 2018. Neural control of startle-induced locomotion by the mushroom bodies and associated neurons in Drosophila. Frontiers in Systems Neuroscience. 12(6).

Young MW. 2000. The tick-tock of the biological clock. Scientific American. 282(3): 64-71.

Vang LL, Medvedev AV, Adler J. 2012. Simple ways to measure behavioral responses of Drosophila to stimuli and use of these methods to characterize a novel mutant. PLOS ONE. 7(5): e37495.

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About the Authors

Caroline Dugan has been part of the Boston University instructional team since 2021, when she began working as an undergraduate Learning Assistant in the NE203 Principles of Neuroscience course. After graduating from Boston University in 2022 with her B.A. in Neuroscience, she became a full-time staff instructor as a Post-Baccalaureate Academic Fellow.

Liam Quidore has been the Neuroscience Lab Supervisor Instructor at Boston University since 2021, where he works to prepare and coordinate all instructional neuroscience labs taught in the undergraduate program.

Dr. Kyle Gobrogge has held the position of Lecturer within the Boston University Undergraduate Program in Neuroscience since 2019. In this capacity, he assumes multiple roles, including Directorship of the NE203 Principles of Neuroscience Laboratory. Beyond this responsibility, he leads the instruction for the advanced elective course Neurobiology of Sex and Aggression, Neuropsychiatry as well as the foundational first-year course Introduction to Neuroscience. Furthermore, Kyle actively guides and advises students who are engaging in independent research pursuits through the Boston University Undergraduate Research Opportunities Program, Kilachand Keystone, and Senior Honor's Thesis.

Dr. John Tullai's background is in Neuroendocrinology and Cancer Biology and has been with the Boston University Undergraduate Program in Neuroscience since 2019. John is the Course Lecturer for NE203 Principles of Neuroscience, and the Director of the NE102 Introduction to Cell and Molecular Biology Laboratories. He likewise teaches upper-level electives including Neural Impacts on Tumorigenesis and new course for 2023-2024, Neurogenetics.

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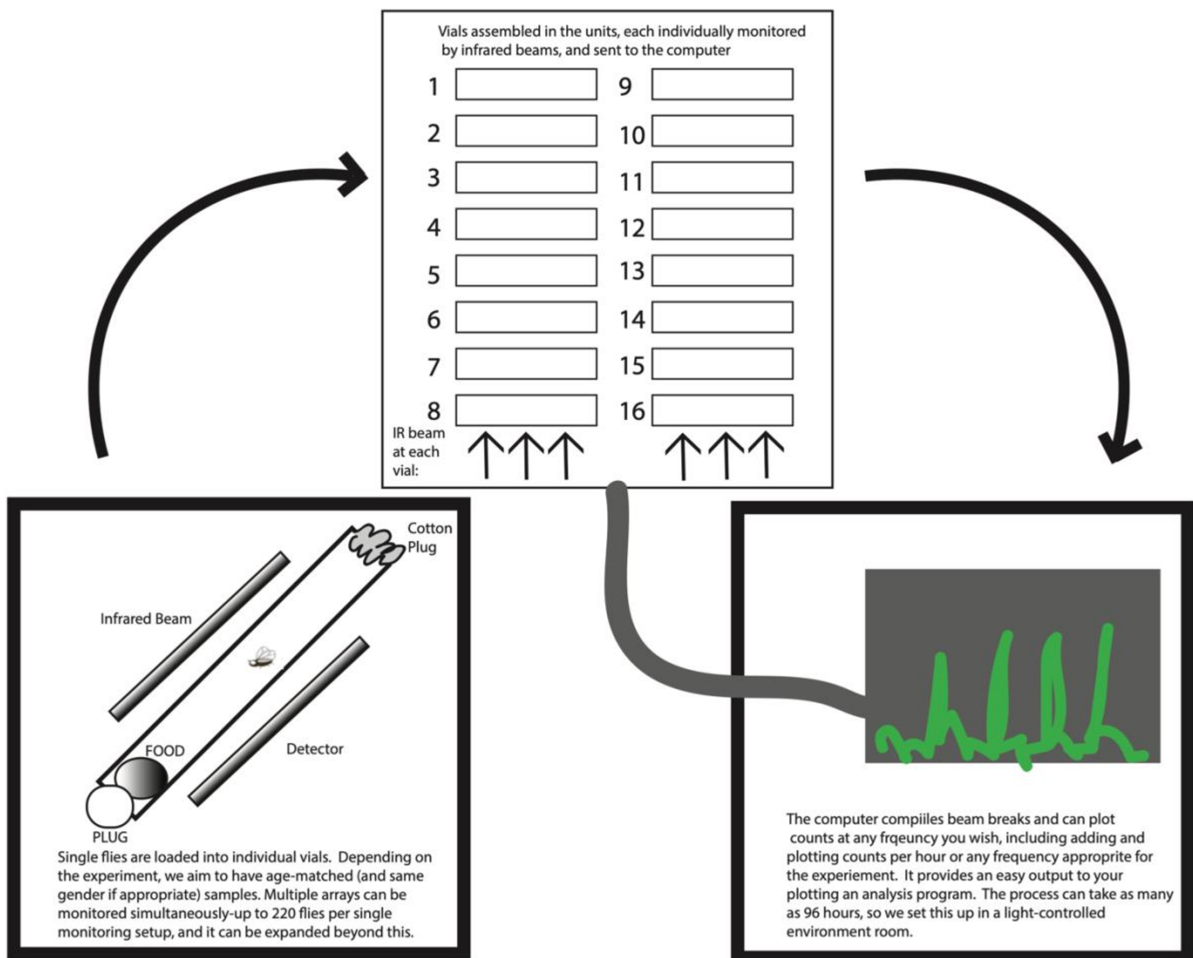
APPENDIX A: STUDENT HANDOUT

NE 203 Fall 2022
Lab 4: Drosophila Activity Monitoring



Monitoring Individual Drosophila Melanogaster Flies and Description of Your Project and Fly Cross Needs

Drosophila Activity Monitors (DAMs):

The 5mm tube diameter is the standard size for measuring the activity of individual flies.

We are using the DAM5H-4 device (Trikinetics, USA), which uses 4 infrared beams, dividing the tube roughly into quadrants. We can record total counts, the position of each count, and the fraction of time spent in each beam over an active monitoring length of 65mm. The DAM5H-4 is capable of characterizing sleep in fast or slowly-moving flies, and can also report the location of their activity for preference assays. The monitor can be used with either horizontally or vertically oriented tubes (for example, for a climbing type assay).

Today, you will also map out specifics of the pathway you are studying, the question being posed, the flies you wish to cross to experiment with, and what your design is to answer the question(s). Please refer to the "DAM Guide" for additional information about the DAMs.

Please watch the DAM Workshop, which will walk you through the different data types and types of assays you may use for your independent projects: https://youtu.be/ypjDH7BL36g . The articles referenced in this video may also be particularly useful for you. (Chiu et al. 2010, Brinson 2022, McParland et al. 2014, Sun et al. 2018, Vang et al. 2012)
You can also access the DAM Workshop slides here:

https://docs.google.com/presentation/d/1OxM_eYXRimmV4w4He9V2pL71Zu-fy9hQhSMUQRb75ZI/edit?usp=sharing.

The DAM5H-4 has 32 positions for vials and IR monitoring, each vial containing 4 sensor points.

TODAY'S TASKS

Part 1: Bioinformatics Dry Lab

Activity 1: Circadian Rhythm Sample Data

1. Today, you will use previously collected sample data to practice using the deconvolution software. Download the **DAMFileScan113X** software: https://trikinetics.com . If you have a Mac, you will likely need to directly allow it to be opened from your security and privacy settings.
2. Access the Monitor1 sample data from here:
   https://drive.google.com/file/d/1bjaqFwF-hNusK3M0aWLKLOkIRYGaNYOv/view?usp=sharing
   a. File names must read as: MonitorNNN.txt or MonitorNNN.csv, where NNN = 1 to 120.
   b. This sample data was collected from students who successfully completed the experiment!
3. Once you have downloaded and saved the sample data, create a folder on your computer that contains ONLY the Monitor1.txt file.
4. Open the Monitor1.txt file in DAMFileScan113X by selecting the folder on your computer that contains the Monitor data text file (Select Input Data Folder).
5. Once you have opened the folder, click "Scan" to verify that the program can read the data.

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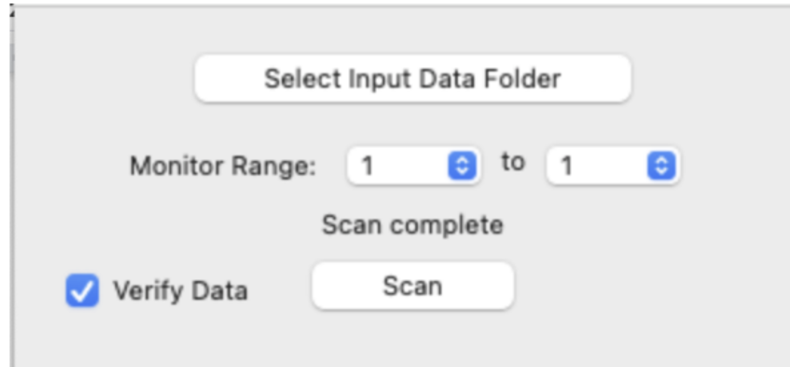
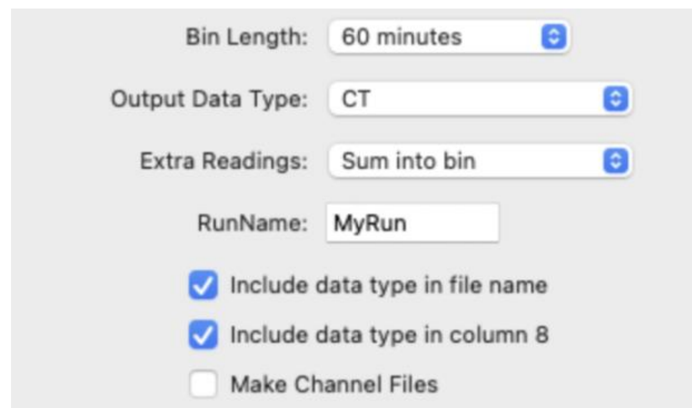
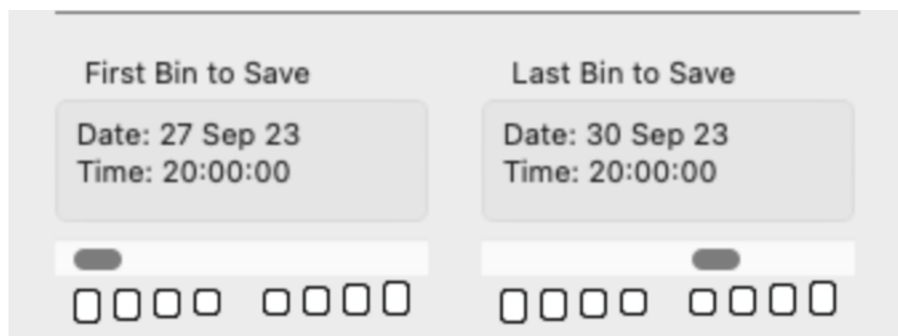


6. For this particular set of sample data, make sure that your settings are as listed below:
   a. Selecting "CT" as your data type will extract only the "count" data from the file. Count data is a running total of how many times a beam of light was broken in the DAM, effectively telling you how much your flies have been moving without worrying about *where* that movement occurred. The sample data was originally collected every 15 seconds for 24 hours - that is a TON of bins! In order to simplify our plots, we are going to look at the data in bins that are an hour long (60 minutes). Since we are interested in how much total movement occurred during that hour, that is why we are going to have the extra readings "summed into bins" instead of "averaged into bins." (If we had clicked average, we would get an AVERAGE count of movement that occurred across that hour rather than total movement.)



7. Next, you will edit the bins to be saved so that you are only examining the time period we are interested in. Using the scroll bars underneath "First Bin to Save" and "Last Bin to Save," set your bins so that we are examining data from September 27, 2023 at 20:00:00 to September 30, 2023 at 20:00:00.



8. Click "Save." This will create a file with the name you entered into RunName in the format MyRun.txt.
9. Open Excel or Google Sheets. Click File → Open → MyRun.txt. You may need to hit "Next" a couple of times until the data opens.
10. You will end up with a fairly large Excel sheet. We are going to plot the activity in each vial separately on one graph, and in another graph, we are going to plot the average activity across all vials. To do this…
11. Open the DAM Data Analysis Template that we have created for you https://drive.google.com/file/d/1IxMWctC5Hynzmwkije5EoMlRtkvfGsHs/view?usp=sharing. Make sure that you download the file and open it in Excel, not Google Sheets. If you have done your settings correctly, you should be able to paste the data from your MyRun file column K into the template under "Vial 1", the data from column L under "Vial 2," the data from column M under "Vial 3," the data from Column N under "Vial 4," and so forth. You can reference the Data Readout Guide to help you keep track of what each column means: https://docs.google.com/spreadsheets/d/1oPR1f9-zWp-cbdPxLDAfLAqpE2KqLURQ/edit?usp=sharing&ouid=103715742842849153088&rtpof=true&sd=true . Once you do this, the Average Activity column should be populated with values, and two graphs should be created for you. It is important to note that "Average Activity" here is the average of the TOTAL counts that we got in each vial. This is different from the average that we saw in the DAMFileScan113X settings. The graph with 32 lines is the activity in each vial (so from each individual fly), and the graph with 1 line is the average activity across vials.
12. From here, you will need to create axis labels, a title, and adjust any formatting you feel is necessary to convey the important information from this data.
13. Please refer to the Trikinetics User Guide for further information about data types and selection: https://trikinetics.com/Downloads/DAM5H%20Data%20Sheet.pdf

Activity 2: Preference Assay Sample Data

1. Now, it is time to work with a different set of data. Instead of examining circadian rhythm, this time, you will be analyzing data from a preference assay.
    a. This experiment examined odor preference in genetically modified flies. On the left side, an unscented cotton ball (neutral odor) was placed in the tube. On the right side, a cotton ball containing diluted peppermint oil (a scent known to be aversive to flies) was placed in the tube.
2. For this experiment, looking at the total movement, or "Count," data is not going to answer the experimental question. Instead, we should be looking for how much time the flies spent on the left side of the tube vs. the right side of the tube.
    a. We will be looking at "Dwell" data this time. Dwell data examines what percentage (0-100%) of time the fly spent in each beam per time bin.
    b. Please refer to the DAM Guide for more detailed information about the data type and codes.
    c. The data code "D12" will provide you with the percentage of time that the fly spent on the left half of the tube for each "bin," or for each time period in which data was collected. The data code "D34" will provide you with the percentage of time that the fly spent on the right half of the tube for each bin.
    d. For this data set, we are ultimately interested in the ***average*** time that the fly spent on each half of the tube for the duration of the experiment.
3. This time, we will provide you with the data set that has been run through DAMFileScan113X to give us "Dwell" data for the left and right half of the tube. You can reference the Data Readout Guide to help you keep track of what each column means: https://docs.google.com/spreadsheets/d/1oPR1f9-zWp-cbdPxLDAfLAqpE2KqLURQ/edit?usp=sharing&ouid=103715742842849153088&rtpof=true&sd=true.
4. Download and open the Preference Assay Sample data in Excel: https://docs.google.com/spreadsheets/d/1wFCDOCNfVFR-TqIVSZLsfY9ZiKi_g7VP/edit?usp=sharing&ouid=103715742842849153088&rtpof=true&sd=true.

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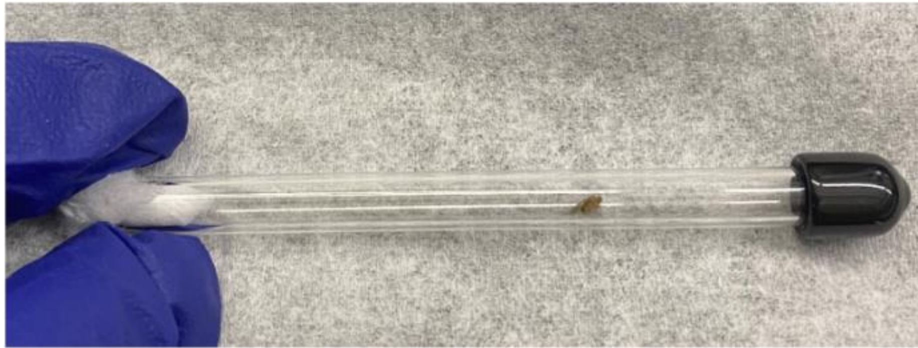
5. This time, it is up to you to make a graph that is representative of the data from the preference assay. We have not provided a template for you. Keep the following information and questions in mind as you create your figure…
   a. The experiment was only run using vials 27, 29, and 31. The rest of the vials can be ignored for this data set.
   b. Are you interested in the dwell data for each bin, or are you interested in the overall average dwell information for the neutral odor vs. aversive odor?
   c. What kind of graph would best represent the comparison between time spent on the left half of the tube vs. time spent on the right half of the tube? Bar, line, pie chart, etc?
   d. What odor does D12 correspond to?
   e. What odor does D34 correspond to?
   f. Your graph labels will be very important for demonstrating the results of the experiment - make sure that the title and axis labels are clear!

Part 2: Invertebrate Handling and Vial Preparation:

You will be provided with vials that already have food and are plugged at the food end. After you capture the fly in the vial, the open end will be plugged with a small amount of cotton.



1. You will receive a vial of wildtype flies from your instructor.
2. Flies will be sedated temporarily using carbon dioxide. Your instructor will demonstrate how to appropriately use the CO_2 gun.
3. Once sedated, we will use MALES ONLY for this exercise. Identify males and transfer them to the DAM vials. Females are not used for circadian rhythm studies, as egg-laying can disrupt monitoring.
4. Plug the open end with cotton. Keep the vials horizontal! While the flies are sedated, if they drop into the food, they will drown and die.
5. You flies will be transported to the DAM units in the 25C environment room and entered into the queue to have their activity monitored for 48-72 hours. Data files will be posted on Blackboard.
6. After practice with the software below, you will then analyze your own flies using the software, and you will use the results in your postlab assignment. Each section will generate 15 flies in vials for the DAMs. Work together!

Part 3: Project Planning

It is time to establish what your project will entail, and in particular, establish what genetic crosses you will conduct. As of now, you have had experience in handling and sexing flies, and have had the genetics toolkit tutorial. Now, it is time to lay down your gameplan. Answer the following questions in detail in your ELN/Spark page:

1. State what pathway you wish to query. (Dopamine, octopamine, serotonin, or acetylcholine). Are you planning to activate or inactivate this pathway? What behavior will you be analyzing?
2. State the hypothesis.
3. How will you answer this question with the flies? Think about what GAL4 and UAS lines you will need for your project. Keep in mind that you will also need to image your neurons of interest.
   a. Go to this link to find out what strains are available: https://docs.google.com/spreadsheets/d/1b8CJ2gPTslXkbmXl2uTGnul40mjR6AVNznJIJg3_j70/edit?usp=sharing.

Electronic Lab Notebook (ELN) Entries:

1. Full DAM setup for your section (image) (**1 point**).
2. Representation of the deconvoluted sample data (one graph for the circadian rhythm data and one for the preference data) (**1 point**).
3. Overview of your group's planned experiment (text) (**1 point**).
4. Explanation of the fly crosses you will be making (with line numbers) (text and/or drawing) (**1 point**)
5. Overview of how you plan to collect behavioral data for your group's experiment (text and/or drawing) (**1 point**)

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APPENDIX B: POSTLAB ASSIGNMENT

NE 203 Fall 2022
Lab 4: Drosophila Activity Monitoring

Postlab Assignment

Part 1: Representation of the Drosophila Activity Monitor Setup (5 points)

1. Turn a photo of the full DAM setup from your section into a figure with an appropriate figure caption.

Part 2: Plot your data using the Excel/Google Sheets Template below. (10 points)

1. Turn the plot of your circadian rhythm data into a figure with an appropriate figure caption.
2. DAM Data Analysis Template: https://drive.google.com/file/d/1IxMWctC5Hynzmwkije5EoMlRtkvfGsHs/view?usp=sharing.

Part 3: Applying your knowledge (5 points)

Answer the following questions based on your knowledge of the DAMs and the data you collected:

1. Looking at the data you collected, were there periods of high activity and periods of low activity? What observations can you make regarding circadian rhythmicity in Drosophila?
2. For this activity, you examined the "count" data type. Imagine you wanted to design a scent preference experiment (meaning you want to investigate whether flies demonstrate a preference for the scent of vanilla or the scent of vinegar, for example).
   a. How would you design this experiment using the Drosophila Activity Monitor?
   b. What data type might you analyze? How would you go about extracting this data from the DAMs?
   c. How might you best represent your findings?
3. Imagine you are interested in designing a climbing assay to test Drosophila locomotion.
   a. How would you design this experiment using the DAM?
   b. What data type might you analyze? How would you go about extracting this data from the DAMs?
   c. How might you best represent your findings?

APPENDIX C: DROSOPHILA ACTIVITY MONITOR TIPS

Please refer to the Trikinetics DAM5H-4 Guide for detailed information about the types of data that can be collected (Trikinetics, USA): https://trikinetics.com/Downloads/DAM5H%20Data%20Sheet.pdf. For your benefit, we have expanded on the information included in the Trikinetics DAM5H Guide below in the hopes that it will help you better understand the types of data you will be collecting.

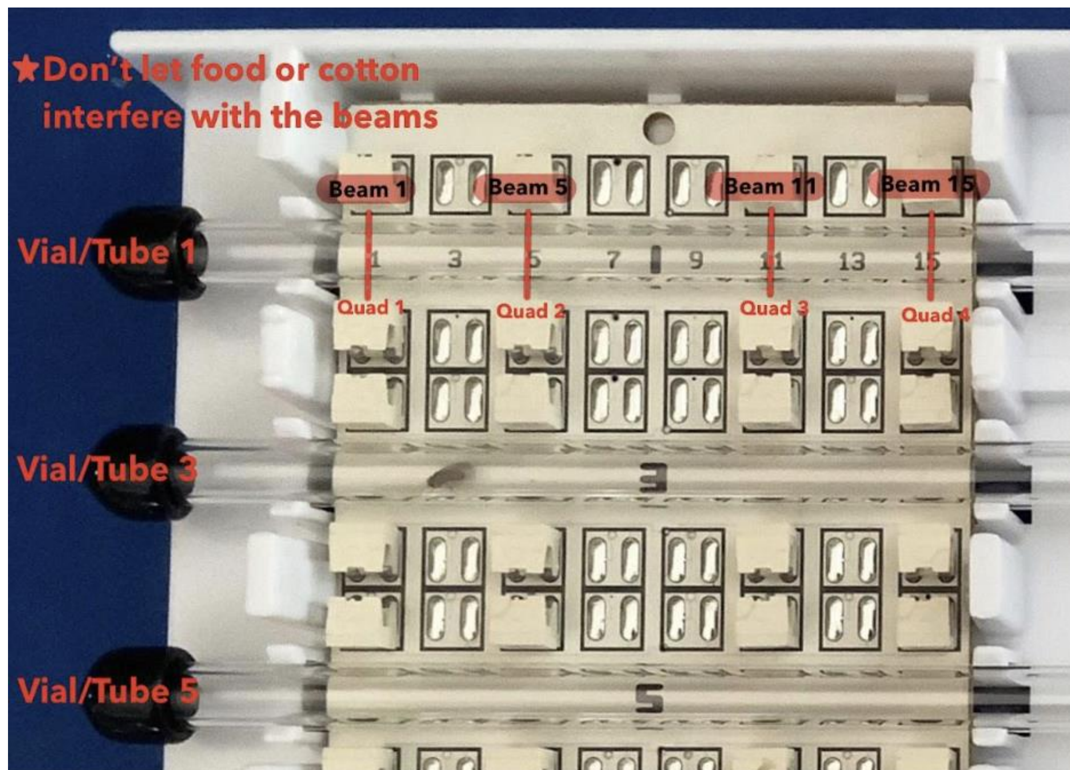
Relevant information to keep in mind:

- Make sure that the cotton, food, and cap do NOT block any of the light beams.
- A "bin" is a way to group data – in the case of the DAMs, the bins are your reading period (i.e., if data is populated to your data file each minute, each minute of data is 1 "bin")
- We have the 4-beam option of the DAMs, so there are four beams labeled 1, 5, 11, and 15.

DAM5 Data Select

Acquire	Save
☐ Moves	☐ Total
☐ Counts	☐ Half
☐ Dwell	☐ Quad
☐ Latency	☐ Beam
☐ Position	
☐ Rest	

Uplink delay: 0.7 sec



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ACQUIRE = data type collected per bin (for example, you can collect count and dwell data at the same time).

MOVES – one "move" is registered when a fly enters a *new beam*. A move is not registered if a fly is active but does not move to a new beam. For instance, if a fly is very active in Beam 1, but never moves to Beam 5, only one move would be registered.

COUNTS – one "count" is registered any time a beam is broken by the fly's movement, regardless of which beam that activity occurs in. This means that multiple "counts" may register from activity in any given beam. If we take the above example in which a fly is very active in Beam 1 but never moves to Beam 5, many "counts" could be registered even if only one "move" is registered.

DWELL – Dwell calculates the percentage of time that the fly spent in a given beam. For instance, if a fly spent 30 seconds out of a one-minute bin in Beam 1, 15 seconds in Beam 5, and 15 seconds in Beam 11, the Dwell readout for that bin would be 50 for Beam 1, 25 for Beam 5, and 25 for Beam 11. Since this is a percentage, adding up the Dwell readout from each beam within a vial should equal 100 (e.g. 50 + 25 + 25 = 100 from the previous example). This is useful for preference assays, where you may be interested in whether a fly prefers one side of the vial over the other. If the Dwell from the left side (Beams 1 and 5) is over 50, that would communicate that the fly spent the majority of its time on the left side of the vial.

LATENCY – Latency calculates the difference in time between the start of the bin and when a beam was broken, measured in quarter-seconds (250 milliseconds). For instance, if a one-minute bin begins and it takes the fly 5 seconds to break a beam (i.e. register a 'count'), the latency readout for that bin might be 20 (5 seconds = 20 quarter-seconds) if you are not interested in the latency for a specific beam. It is possible to measure the latency of each beam individually. In a one-minute bin, if it takes the fly 5 seconds to break Beam 1 but thirty seconds to break Beam 11, the latency for Beam 1 would be 20 (5 seconds = 20 quarter-seconds), but the latency for Beam 11 would be 120 (30 seconds = 120 quarter-seconds).

POSITION – Position tells you the most recent beam that registered fly activity. In other words, which beam was last broken. In the 4-beam unit, the beams are numbered 1, 5, 11, and 15. Thus, if the number in the position column was 1, that means the fly most recently entered Beam 1.

REST – Rest is a continuous value which measures how long it has been since activity was recorded for a fly. When a move is registered in the DAM, rest goes back to zero. "Rest" data can get larger and larger across many bins if a fly has not moved for quite some time. For instance, if you are recording data every minute, but a fly is stationary for ten minutes, the value of rest would get larger across those ten bins until the fly entered a new beam again, when it would be reset to zero. Larger numbers for "rest" tell you that there has been a period of inactivity. Smaller numbers for rest may indicate that the fly is active (i.e. the fly was not motionless for an extended period of time).

SAVE = how you will collect the data you selected (only applies to move, count, dwell, and latency).

TOTAL – Generates a total sum of moves or counts in a given tube, without regard to positioning. For instance, in Tube 1, Moves total would be the sum of all moves recorded from Beam 1, 5, 11, and 15 in Tube 1.

HALF – generates a sum of moves, counts, or dwell data broken up into the left and right side of the tube. For instance, in tube 1, Moves Half would give you the total sum of all moves recorded from Beams 1 and 5 (the left half) and the total sum of all moves recorded from Beams 11 and 15 (the right half). If latency is selected along with half, the latency for Beams 1 and 15 will be reported.

QUAD – generates a sum of moves, counts, or dwell data broken up into the four quadrants of the tube. If Latency is chosen with quad, the latency of each beam will be given for the four-beam model (see LATENCY above for more

information). With the four-beam device, this will essentially give you a readout of what is occurring at each beam. For instance, Moves Quad would give you the moves recorded from Quad 1 (Beam 1), Quad 2 (Beam 5), Quad 3 (Beam 11), and Quad 4 (Beam 15).

BEAM – this data type might be selected for an assay in which you connect two tubes end to end and span across two rows (for example, you put a tube in spots 1 and 2, pushed them end-to-end, and allowed the fly to have the full width of the board. When "Beam" is selected, four rows of data will be created per tube per bin, giving you the count and move data for the adjacent tubes (tubes 1 and 2 for example). For the purposes of our course, most students will not need to use the Beam option given that we use the four-beam version of the DAM.

DATA CODES

Column 8 of your data file tells you the data type according to the Data Code listed below. Using the DAMFileScan113X application, you can filter your data to only examine one type at a time.

MT - Moves Total

This takes the total sum of moves from every beam in each tube for that bin (Moves in Beam 1 + Moves in Beam 5 + Moves in Beam 11 + Moves in Beam 15 = MT).

M12 – Moves Half (Left)

The sum of moves on the left side of the tube. In other words, this takes the sum of moves from beams located only on the left side of the tube (Moves in Beam 1 + Moves in Beam 5 = M12).

M34 – Moves Half (Right)

The total sum of moves on the right side of the tube. In other words, this takes the sum of moves from beams located only on the right side of the tube. (Moves in Beam 11 + Moves in Beam 15 = M34).

M1 – Moves Quad 1

The total sum of moves in Quadrant 1 of the tube. This takes the sum of moves from beams located in Quadrant 1 (Moves in Beam 1 = M1).

M2 – Moves Quad 2

The total sum of moves in Quadrant 2 of the tube. This takes the sum of moves from beams located in Quadrant 2 (Moves in Beam 5 = M2).

M3 – Moves Quad 3

The total sum of moves in Quadrant 3 of the tube. This takes the sum of moves from beams located in Quadrant 3 (Moves in Beam 11 = M3).

M4 – Moves Quad 4

The total sum of moves in Quadrant 4 of the tube. This takes the sum of moves from beams located in Quadrant 4 (Moves in Beam 15 = M4).

CT - Counts Total

This takes the total sum of counts from every beam in a given tube for that bin (Counts in Beam 1 + Counts in Beam 5 + Counts in Beam 11 + Counts in Beam 15 = CT).

C12 – Counts Half (Left)

The sum of counts on the left side of the tube. In other words, this takes the sum of counts from beams located only on the left side of the tube (Counts in Beam 1 + Counts in Beam 5 = C12).

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C34 – Counts Half (Right)
The sum of counts on the right side of the tube. In other words, this takes the sum of counts from beams located only on the right side of the tube (Counts in Beam 11 + Counts in Beam 15 = C34).

C1 – Counts Quad 1
The sum of counts in Quadrant 1 of the tube. This takes the sum of counts from beams located in Quadrant 1 (Counts in Beam 1 = C1).

C2 – Counts Quad 2
The sum of counts in Quadrant 2 of the tube. This takes the sum of counts from beams located in Quadrant 2 (Counts in Beam 5 = C2).

C3 – Counts Quad 3
The sum of counts in Quadrant 3 of the tube. This takes the sum of counts from beams located in Quadrant 3 (Counts in Beam 11 = C3).

C4 – Counts Quad 4
The sum of counts in Quadrant 4 of the tube. This takes the sum of counts from beams located in Quadrant 4 (Counts in Beam 15 = C4).

D12 – Dwell Half (Left)
The sum of dwell data from the left side of the tube. This takes the sum of dwell data from beams located on the left side of the tube (Dwell in Beam 1 + Dwell in Beam 5 = D12).

D34 – Dwell Half (Right)
The sum of dwell data from the right side of the tube. This takes the sum of dwell data from beams located on the right side of the tube (Dwell in Beam 11 + Dwell in Beam 15 = D34).

D1 – Dwell Quad 1
The sum of dwell data in Quadrant 1 of the tube. This takes the sum of dwell data from beams located in Quadrant 1 (Dwell in Beam 1 = D1).

D2 – Dwell Quad 2
The sum of dwell data in Quadrant 2 of the tube. This takes the sum of dwell data from beams located in Quadrant 2 (Dwell in Beam 5 = D2).

D3 – Dwell Quad 3
The sum of dwell data in Quadrant 3 of the tube. This takes the sum of dwell data from beams located in Quadrant 3 (Dwell in Beam 11 = D3).

D4 – Dwell Quad 4
The sum of dwell data in Quadrant 4 of the tube. This takes the sum of dwell data from beams located in Quadrant 4 (Dwell in Beam 15 = D4).

L12 – Latency Half (Left)
Records how long it took the fly to break Beam 1 after the bin started (Latency of Beam 1 = L12).

L34 – Latency Half (Right)
Records how long it took the fly to break Beam 15 after the bin started (Latency of Beam 15 = L34).

L1 – Latency Quad 1
Records how long it took the fly to break Beam 1 after the bin started (Latency of Beam 1 = L1).

L2 – Latency Quad 2
Records how long it took the fly to break Beam 5 after the bin started (Latency of Beam 5 = L2).

L3 – Latency Quad 3
Records how long it took the fly to break Beam 11 after the bin started (Latency of Beam 11 = L3).

L4 – Latency Quad 4
Records how long it took the fly to break Beam 15 after the bin started (Latency of Beam 15 = L4).

Pn – Position (see description above)

Rt – Rest (see description above)

NOTE: You can choose more than one data readout type. For example, you could choose count and position. For each time period, in this case, you would have two rows of data: one would correspond to count and one would correspond to position.

Click the link below for a sample excel Sheet that contains a guide to the data in each column.
https://docs.google.com/spreadsheets/d/1oPR1f9-zWp-cbdPxLDAfLAqpE2KqLURQ/edit?usp=sharing&ouid=103715742842849153088&rtpof=true&sd=true

A few details to keep in mind:

- If you chose more than one data type to be measured during your experiment (position and count, for example), you may have two rows for each index. This means that, for each time period the DAM collected data, it will give you an output for both data types.
- Depending on your settings, your data may look slightly different. By using the Data Readout Guide and the above instructions, you should be able to decipher what you're seeing.
- In the "Monitor Status" column, a value of 1 indicates that valid data was collected. A value of 50 or 51 indicates that no data was collected.

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