Protocol

Analysis of Sleep and Circadian Rhythms from *Drosophila* Activity-Monitoring Data Using SCAMP

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Sleep is a fundamental feature of life for virtually all multicellular animals, but many questions remain about how sleep is regulated and what biological functions it plays. Substantial headway has been made in the study of both circadian rhythms and sleep in the fruit fly *Drosophila melanogaster*, much of it through studies of individual fly activity using beam break counts from *Drosophila* activity monitors (DAMs). The number of laboratories worldwide studying sleep in *Drosophila* has grown from only a few 20 years ago to hundreds today. The utility of these studies is limited by the quality of the metrics that can be extracted from the data. Many software options exist to help analyze DAM data; however, these are often expensive or have significant limitations. Therefore, we describe here a method for analyzing DAM-based data using the sleep and circadian analysis MATLAB program (SCAMP). This user-friendly software has an advantage of combining several analyses of both sleep and circadian rhythms in one package and produces graphical outputs as well as spreadsheets of the outputs for further statistical analysis. The version of SCAMP described here is also the first published software package that can analyze data from multibeam DAM5Ms, enabling determination of positional preference over time.

MATERIALS

Equipment

DAMFileScan software (TriKinetics)

Data-analysis computer (Macintosh or PC)

Data collected from DAMSystem3 software [see Protocol: **Neural Stimulation During *Drosophila* Activity Monitor (DAM)-Based Studies of Sleep and Circadian Rhythms in *Drosophila melanogaster*** (Vecsey et al. 2024) and Protocol: **Analysis of Positional Preference in *Drosophila* Using Multibeam Activity Monitors** (Porter et al. 2024)]

Excel (Microsoft)

MATLAB (MathWorks, any version since 2010)

Sleep and circadian analysis MATLAB program (SCAMP)

SCAMP_v4 is recommended (available freely from Chris Vecsey: https://academics.skidmore.edu/blogs/cvecsey/?page_id=57).

Statistical analysis software

We use JMP Pro 15 and GraphPad, but many kinds would suffice.

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From the *Drosophila* Neurobiology collection, edited by Bing Zhang, Ellie Heckscher, Alex C. Keene, and Scott Waddell.

Advanced Online Article. Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot108182

METHOD

1. On the computer, download and install DAMFileScan (the newest version is available at https://www.trikinetics.com), MATLAB (https://www.mathworks.com), and SCAMP. When setting up MATLAB, make sure that the "Signal Processing" toolbox is included in the installation.

   This toolbox contains a filtering script called "butter.m" that is required for the analysis of circadian rhythmicity.

2. Once SCAMP is downloaded, move the entire folder to the MATLAB folder (on Macs, this is typically in the Documents folder). Within MATLAB, use "Set Path" to select the SCAMP folder (including all subfolders).

   This allows MATLAB to find all scripts needed for SCAMP to operate.

3. Use the DAMFileScan program to create individual data files for each fly.

   The SCAMP program will need to access two folders containing individual data files binned in 1-min and 30-min increments. Use identical file names in each folder; only the names of the folders themselves should differ.

4. If an environmental monitor was included in the incubator with the *Drosophila* activity monitors (DAMs) (this is highly recommended), run the script to graph environmental monitor data by simultaneously selecting the 1-min data files from channels 04, 09, and 14 from the environmental monitor and typing "EnvMonData" in the MATLAB command window.

   The capitalization is important! This will allow visualization of the light intensity, temperature, and humidity level inside the incubator throughout the experiment (see Fig. 1). We recommend performing this step first before going forward with SCAMP analysis.

5. Run SCAMP within MATLAB.
   - i. Type "scamp" in the MATLAB command window.

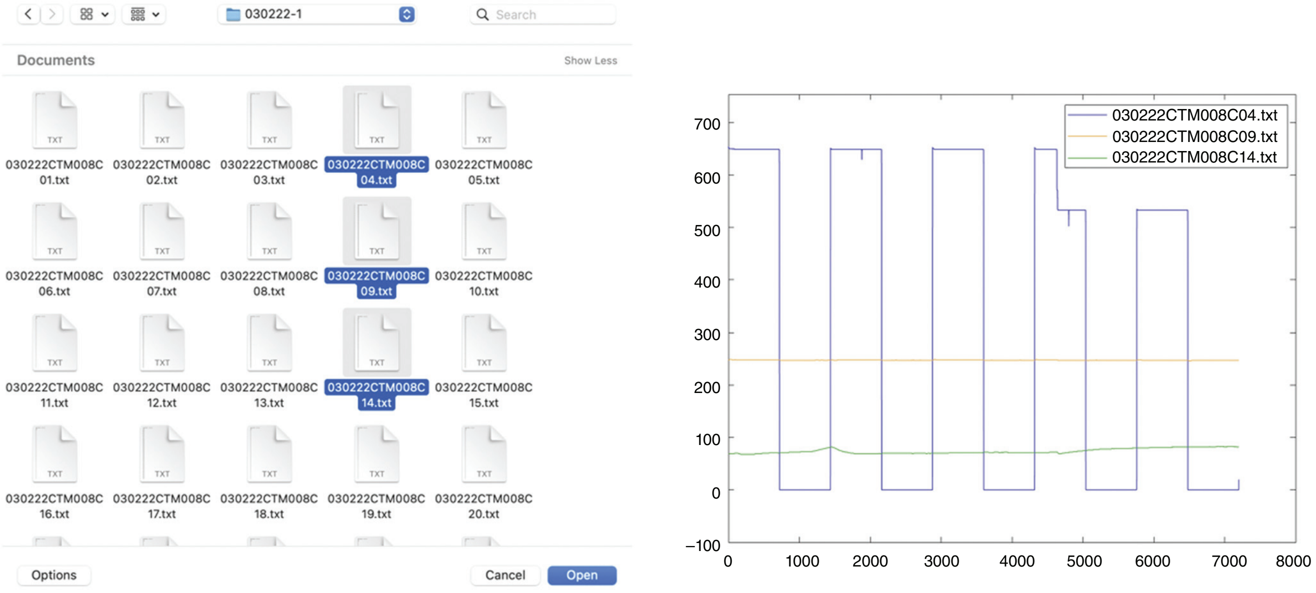
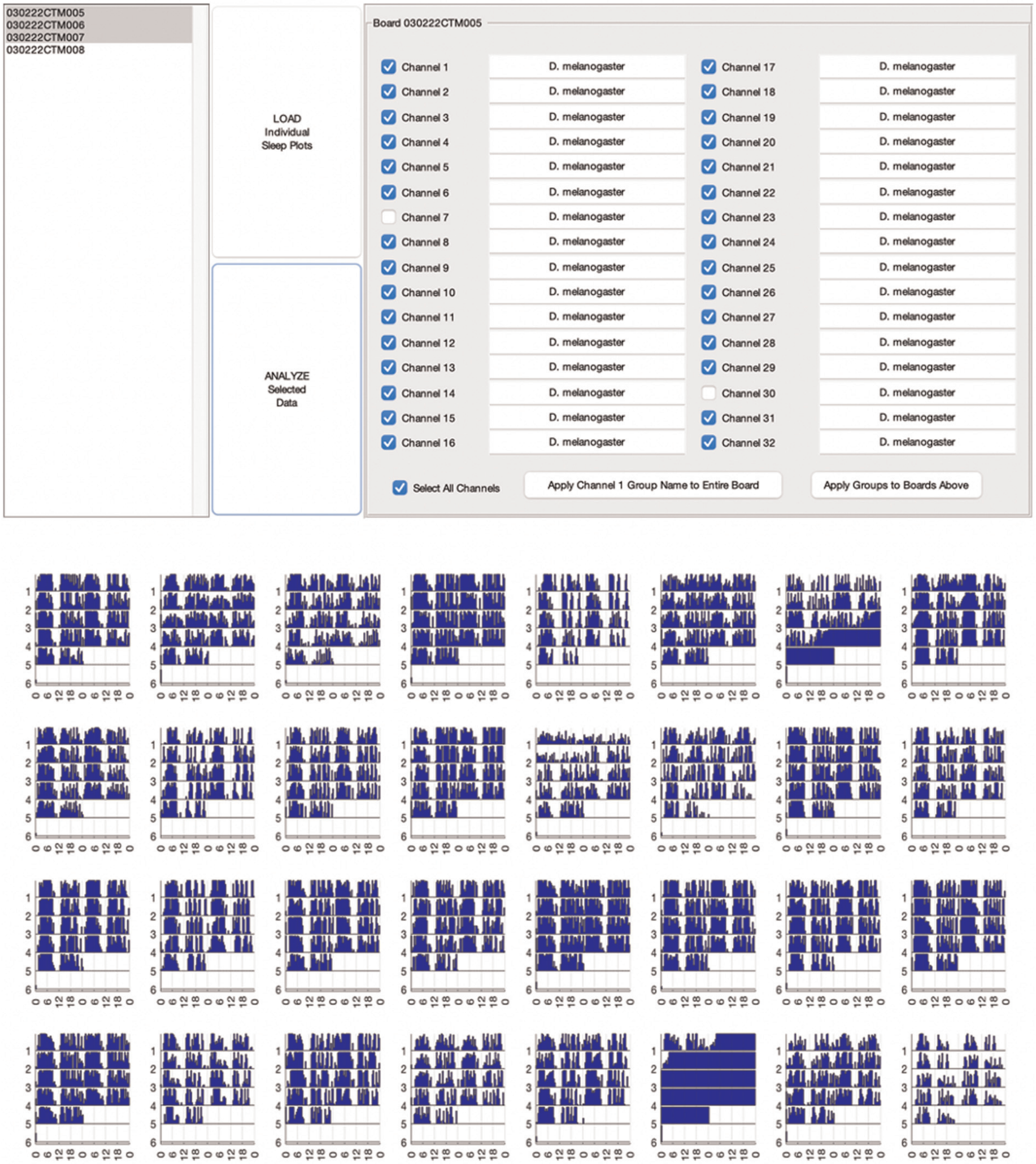


FIGURE 1. Environmental monitor analysis. (*Left*) This file-selection window was generated by running the "EnvMonData" script within the MATLAB-based SCAMP analysis package. Simultaneously selecting channels 04, 09, and 14 from the 1-min data from the environmental monitor (in this case, M008) and clicking "Open" created the output graph on the *right*. (*Right*) Output graph showing light intensity in blue (in lux, C04), temperature in orange (in Celsius × 10; thus, a value of 250 on the graph represents 25.0°C, C09), and humidity in green (in percent, C14). The *x*-axis is in minutes (there are 1440 min/d). In this experiment, there were five 12-h–12-h light–dark days, in which the light intensity was reduced during the 4th day from 650 to 550 lux.

ii. Select the folder containing the 1-min data and then select the folder containing the 30-min data.

This will open the "Fly Selection Window" (see Fig. 2), where group names can be assigned and dead/unwanted individual flies and entire monitors can be excluded from subsequent analysis.



030222CTM005
030222CTM006
030222CTM007
030222CTM008

LOAD Individual Sleep Plots

ANALYZE Selected Data

Board 030222CTM005

☑ Channel 1	D. melanogaster	☑ Channel 17	D. melanogaster
☑ Channel 2	D. melanogaster	☑ Channel 18	D. melanogaster
☑ Channel 3	D. melanogaster	☑ Channel 19	D. melanogaster
☑ Channel 4	D. melanogaster	☑ Channel 20	D. melanogaster
☑ Channel 5	D. melanogaster	☑ Channel 21	D. melanogaster
☑ Channel 6	D. melanogaster	☑ Channel 22	D. melanogaster
☐ Channel 7	D. melanogaster	☑ Channel 23	D. melanogaster
☑ Channel 8	D. melanogaster	☑ Channel 24	D. melanogaster
☑ Channel 9	D. melanogaster	☑ Channel 25	D. melanogaster
☑ Channel 10	D. melanogaster	☑ Channel 26	D. melanogaster
☑ Channel 11	D. melanogaster	☑ Channel 27	D. melanogaster
☑ Channel 12	D. melanogaster	☑ Channel 28	D. melanogaster
☑ Channel 13	D. melanogaster	☑ Channel 29	D. melanogaster
☑ Channel 14	D. melanogaster	☐ Channel 30	D. melanogaster
☑ Channel 15	D. melanogaster	☑ Channel 31	D. melanogaster
☑ Channel 16	D. melanogaster	☑ Channel 32	D. melanogaster

☑ Select All Channels

FIGURE 2. Fly-selection window and sleep plot window. (*Top*) The first major step in running SCAMP analysis is to assign group names to each fly (here all flies are labeled "*D. melanogaster*") and select dead flies by selecting a monitor on the *left* and clicking the "LOAD Individual Sleep Plots" button to generate the graph at the *bottom*. (*Bottom*) This output shows sleep amounts in blue in double-plotted graphs for each fly in a monitor (channels 1–8 on the *top* row, channels 9–16 on the second row, etc.). In this case of a 5-d experiment, flies 7 and 30 appear to have died before the end of the experiment, based on the fact that they had extended periods without any activity (solid blue bar).

iii. Assign group names.

iv. Identify dead flies by running the "LOAD Individual Sleep Plots" function for each monitor and examining the data to look for flies that did not cross the beam for an extended period of time.

This will appear as a block of uninterrupted sleep.

v. If necessary, exclude flies by deselecting them.

vi. When finished, choose all the monitors for further analysis and then click "ANALYZE Selected Data."

6. When the "Choose Analysis" window opens (see Fig. 3), choose the bin length to analyze and click "Analyze for Chosen Bin."

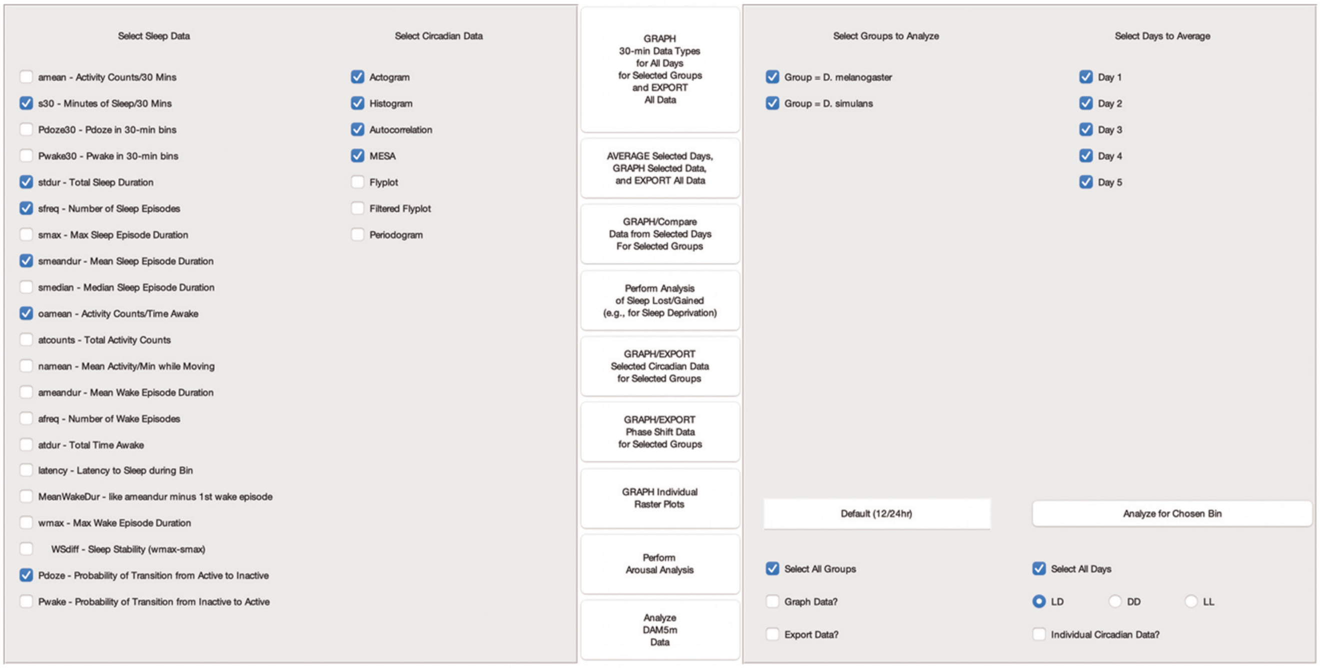
   The default option is to analyze over each 24-h period and also over the first 12 h (assumed to be the light period) and the last 12 h (assumed to be the dark period) of each 24-h period. However, one of the advantages of SCAMP is that it allows the user to choose other bin lengths as long as they divide evenly into 24 h. Therefore, bin lengths of 1, 2, 3, 4, 6, and 8 h are also available. This can be useful when the user wishes to assess sleep responses during a short period of manipulation or when changes in sleep are limited to a specific period.

7. Select groups and days of interest, as well as whether graphical and/or data table outputs are desired, and then run analysis types of choice by clicking a button in the central column (see Fig. 3) (another advantage of SCAMP is that it consolidates several forms of analysis of both sleep and circadian rhythms into one package). From the top to the bottom in SCAMP_v4, the analysis buttons carry out the following actions:

   - Create 30-min binned plots of sleep for each day of the experiment.

     This is useful for seeing overall trends in the data.

   - For user-selected bin length, average user-selected days and plots from user-selected analyses from the list on the left side of the window.



Select Sleep Data

☐ amean - Activity Counts/30 Mins
☑ s30 - Minutes of Sleep/30 Mins
☐ Pdoze30 - Pdoze in 30-min bins
☐ Pwake30 - Pwake in 30-min bins
☑ stdur - Total Sleep Duration
☑ sfreq - Number of Sleep Episodes
☐ smax - Max Sleep Episode Duration
☑ smeandur - Mean Sleep Episode Duration
☐ smedian - Median Sleep Episode Duration
☑ oamean - Activity Counts/Time Awake
☐ atcounts - Total Activity Counts
☐ namean - Mean Activity/Min while Moving
☐ ameandur - Mean Wake Episode Duration
☐ afreq - Number of Wake Episodes
☐ atdur - Total Time Awake
☐ latency - Latency to Sleep during Bin
☐ MeanWakeDur - like ameandur minus 1st wake episode
☐ wmax - Max Wake Episode Duration
☐ WSdiff - Sleep Stability (wmax-smax)
☑ Pdoze - Probability of Transition from Active to Inactive
☐ Pwake - Probability of Transition from Inactive to Active

Select Circadian Data

☑ Actogram
☑ Histogram
☑ Autocorrelation
☑ MESA
☐ Flyplot
☐ Filtered Flyplot
☐ Periodogram

Select Groups to Analyze

☑ Group = D. melanogaster
☑ Group = D. simulans

Select Days to Average

☑ Day 1
☑ Day 2
☑ Day 3
☑ Day 4
☑ Day 5

☑ Select All Groups
☐ Graph Data?
☐ Export Data?

☑ Select All Days
● LD ○ DD ○ LL
☐ Individual Circadian Data?

FIGURE 3. Main analysis window in SCAMP. This window allows users to access all analysis options available in SCAMP. The first step is to select a bin length (currently the default of 12/24 h is selected) and click "Analyze for Chosen Bin." Next, the desired groups and days to analyze can be selected, and any of the buttons in the center column can be pressed to run that particular analysis. In addition, options are available for many of the analyses to create graphical outputs or export the results in Excel-compatible formats.

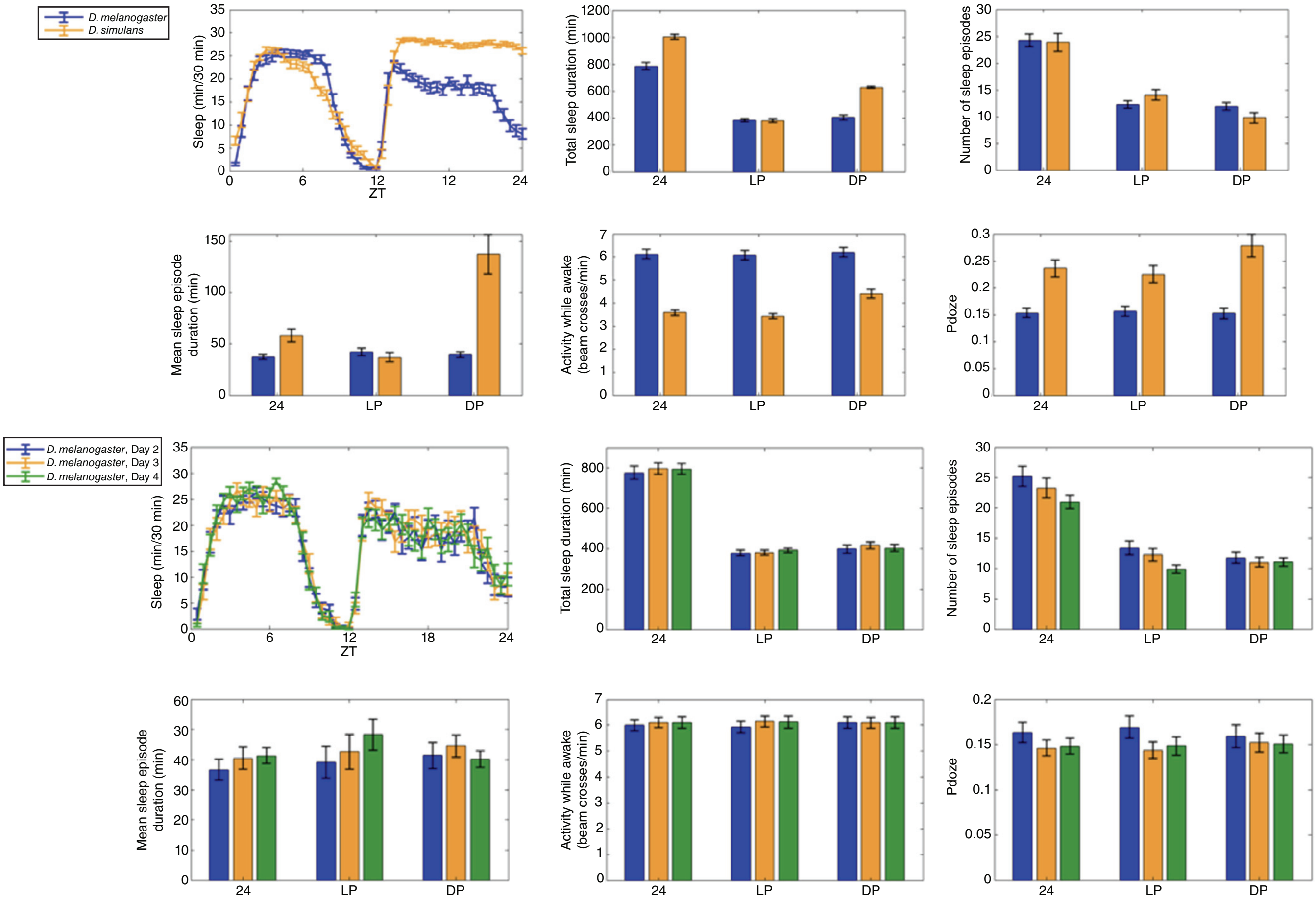


FIGURE 4. SCAMP-generated examples of sleep parameter graphs. (*Top*) Group averages over user-selected days are plotted for six user-selected sleep parameters (sleep/30-min bin, total sleep, number of sleep episodes, mean sleep episode duration, activity/minute while awake, and probability of falling asleep [Pdoze]). (*Bottom*) The same six parameters are compared across three successive days in the same group.

This is useful for between-group comparisons of specific sleep parameters (see Fig. 4, top).

- For user-selected bin length, create graphs of user-selected groups over user-selected days.

  This is particularly useful for examining how a group's sleep parameters change over successive days (see Fig. 4, bottom).

- Open a separate window in which the user can choose specific days and times over which to compare sleep. Perform subtractions cumulatively, where sleep lost or gained builds over successive 30-min periods (see Fig. 5, left), or noncumulatively, where each 30-min period is compared separately between days (see Fig. 5, right).

  This is useful for any situation in which the user wishes to calculate changes in sleep between one condition and another.

- For user-selected days and groups, create graphs of user-selected circadian output data types. Select the "Individual Circadian Data" checkbox to make additional output options available, including the ability for the user to select which cutoff they would like to use for characterizing each fly as rhythmic or arrhythmic.

  These analyses are useful for examining the strength of circadian rhythmicity and period length (see Fig. 6).

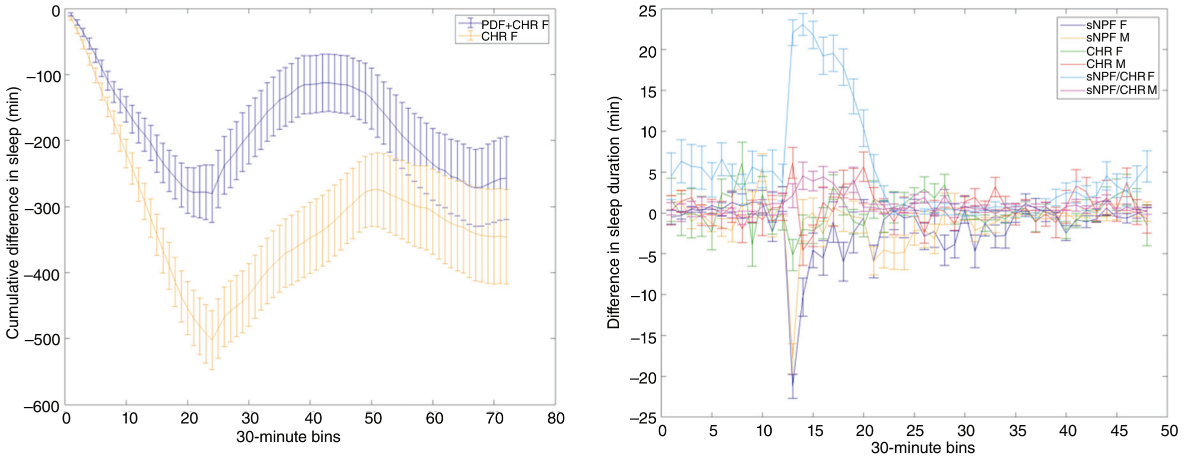
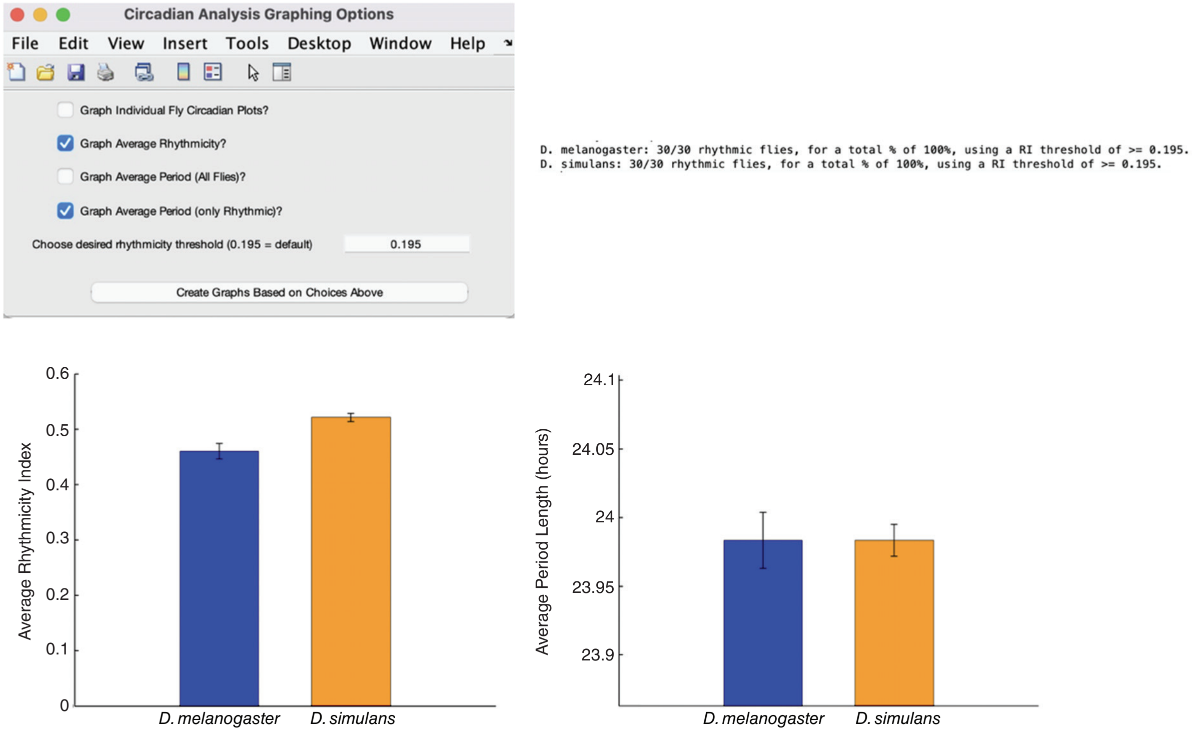


FIGURE 5. SCAMP-generated example of sleep subtraction graphs. (*Left*) A cumulative sleep subtraction, in which total changes in sleep compared with a baseline period are tabulated across the user-specified experimental period. In this case, sleep loss during a 12-h period of sleep deprivation and a subsequent 24-h period of rebound sleep can be seen in two groups: one (PDF + CHR) in which a PDF-GAL4 driver is controlling expression of the optogenetic red-light sensor Chrimson, and another genetic control (CHR) in which no driver was present. This type of graph shows clearly that the control group lost more sleep during the deprivation period but that both groups rebounded in similar ways. (*Right*) A 24-h noncumulative sleep subtraction showing how sleep in each 30-min bin during the experimental period compared with the same time point during the baseline period. In this case, the "sNPF/CHR F" group represents female flies expressing CHR under the control of an sNPF-GAL4 driver. Red-light stimulation was given for 1 h at circadian time (CT)7, and this type of graph shows clearly the resulting substantial and long-lasting increase in sleep compared to the baseline day, a finding similar to what has been published previously (Juneau et al. 2019). (PDF) Pigment dispersing factor, (sNPF) short neuropeptide F.



Circadian Analysis Graphing Options

☐ Graph Individual Fly Circadian Plots?

☑ Graph Average Rhythmicity?

☐ Graph Average Period (All Flies)?

☑ Graph Average Period (only Rhythmic)?

Choose desired rhythmicity threshold (0.195 = default) 0.195

```
D. melanogaster: 30/30 rhythmic flies, for a total % of 100%, using a RI threshold of >= 0.195.
D. simulans: 30/30 rhythmic flies, for a total % of 100%, using a RI threshold of >= 0.195.
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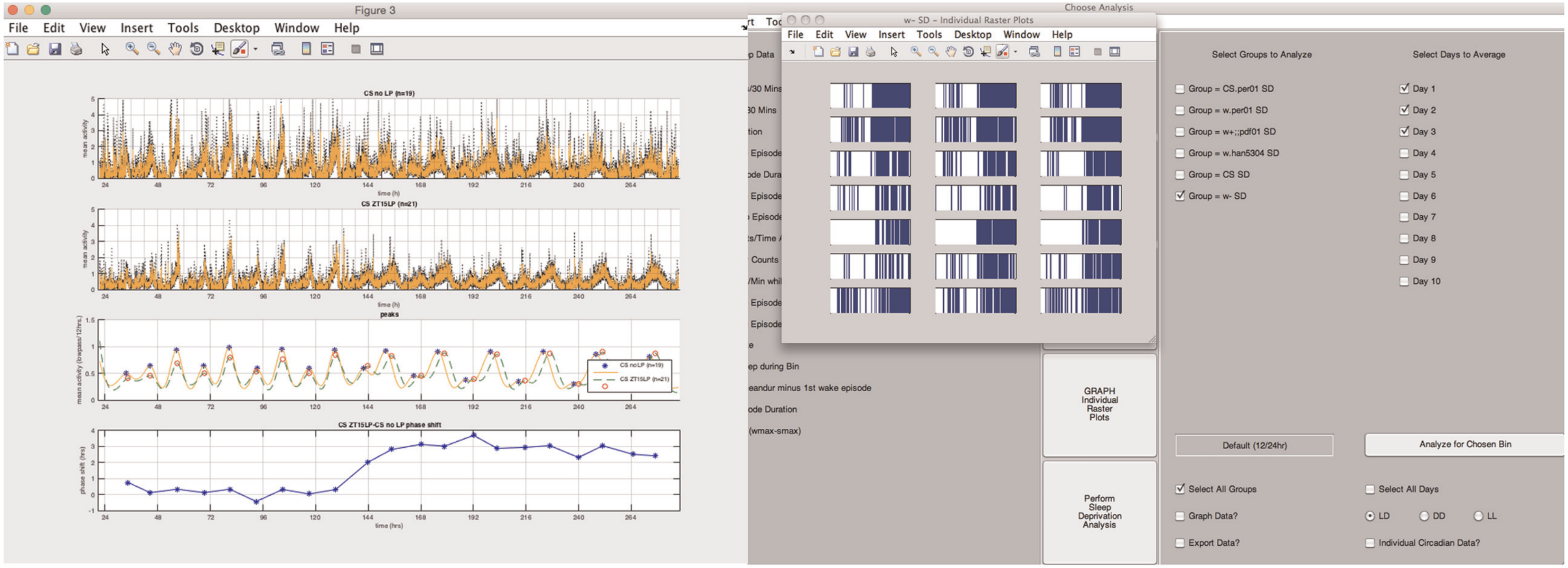
FIGURE 6. Circadian analysis dialog box and resulting outputs. (*Top left*) Dialog box in which the user can choose which outputs are generated and which value to use as the threshold for rhythmicity (0.195 is the default). (*Top right*) Textual output from the MATLAB command window stating the numbers of rhythmic flies out of the total numbers of flies in each group, and which threshold was used. (*Bottom left*) Average rhythmicity bar graph showing rhythmicity index values averaged across all flies in each group. (*Bottom right*) Period-length bar graph, averaged across only the rhythmic flies. Alternatively, the user can choose in the dialog box to calculate period length across all flies instead.



Select Groups to Analyze

☐ Group = CS.per01 SD
☐ Group = w.per01 SD
☐ Group = w+;;pdf01 SD
☐ Group = w.han5304 SD
☐ Group = CS SD
☑ Group = w- SD

Select Days to Average

☑ Day 1
☑ Day 2
☑ Day 3
☐ Day 4
☐ Day 5
☐ Day 6
☐ Day 7
☐ Day 8
☐ Day 9
☐ Day 10

☑ Select All Groups
☐ Graph Data?
☐ Export Data?
☐ Select All Days
● LD ○ DD ○ LL
☐ Individual Circadian Data?

FIGURE 7. SCAMP-generated examples of phase shift and raster plot graphical outputs. (*Left*) Output graph showing a relative phase shift observed between the two groups, one of which was phase-delayed by receiving a 15-min light pulse (LP) early during the dark period (CS ZT15 LP), whereas the other received no such light pulse (CS NoLP). The LP occurred at ZT15, 3 h after dark onset, on the fifth day. Following that, both groups were shifted into total darkness for the remainder of the experiment. The *bottom* two graphs show that this protocol caused the group receiving the LP to shift their activity rhythms later in the day. The *bottom* plot in particular provides quantitative differences in timing between the two groups. (*Right*) raster plot output showing that six flies from one particular group had individually consistent sleep patterns (minutes of sleep shown in blue) over three successive days of data (*left* to *right*). These raster plots are useful ways to visualize individual fly responses to manipulations but do not generate quantitative data. (CS) Canton-S (wild-type flies), (ZT) zeitgeber time.

- A more specialized analysis type: Calculate phase shifts in activity peaks between pairs of groups.

  This is useful for assessing the ability of a fly line to undergo a circadian phase shift relative to a control (see Fig. 7, left).

- For user-selected groups, create raster plots for each individual fly showing the timing of sleep across each user-selected day.

  This is useful for graphical visualization of sleep patterns among individual flies (see Fig. 7, right).

- Open a separate window in which the user can select the number and times of specific arousal events and the groups to analyze.

  The analysis then calculates changes in activity from before to after each arousal event and the percentage of sleeping flies that were awoken by each event. It also generates Excel-compatible data tables with the results. This is useful for determining how easily different fly lines can be aroused from sleep and for tracking how active they become afterward.

- Run a specialized analysis function for users who collected data using DAM5Ms, which have four beam locations instead of one in the center.

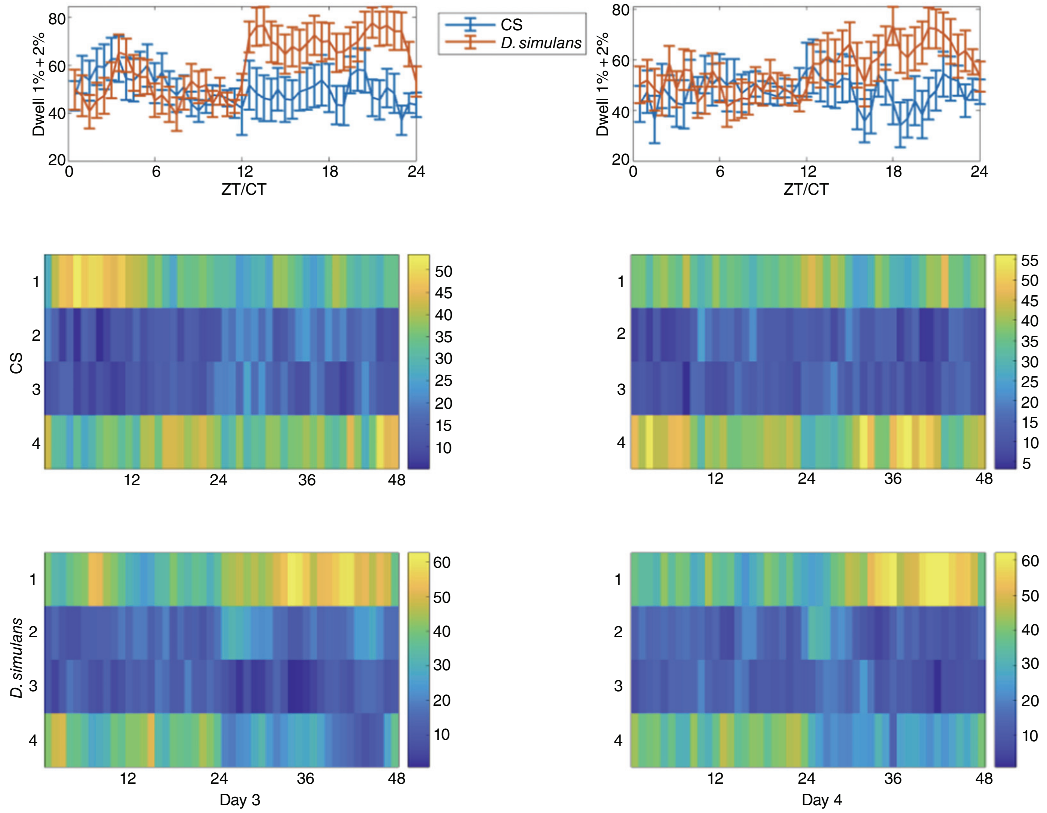
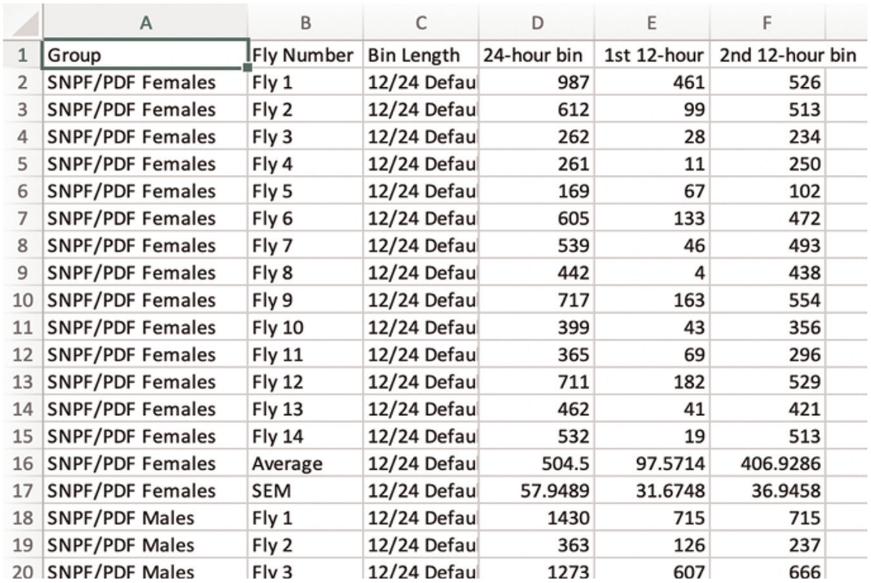


FIGURE 8. SCAMP-generated output graphs from four-beam monitor data analysis. (*Top*) An example of a daily line graph output (in this case, for "Dwell 1 + 2," representing how much time the flies spent on the left side of the tube, closer to beam 1). In this experiment, 0% indicates more time spent near the control food substrate, while 100% represents more time spent near the experimental food substrate. (*Bottom*) An example of heat map plots of time spent (percent for each 30-min bin) in each compartment for each group (rows) and day (columns). Cooler colors represent low percentage values, whereas warmer colors represent high percentage values. Overall, these two graphs can illustrate whether flies from different groups have a preference for one side of the tube compared with the other, and whether that preference changes across the day or during experimental manipulations. Users can select which days to graph. In this example, days 3 and 4 from a multiday experiment are shown for each group in both the line graphs and the heat map plots. (ZT) Zeitgeber time, (CT) circadian time, (CS) Canton-S, a commonly used wild-type strain of *Drosophila melanogaster*.



	A	B	C	D	E	F
1	Group	Fly Number	Bin Length	24-hour bin	1st 12-hour	2nd 12-hour bin
2	SNPF/PDF Females	Fly 1	12/24 Defau	987	461	526
3	SNPF/PDF Females	Fly 2	12/24 Defau	612	99	513
4	SNPF/PDF Females	Fly 3	12/24 Defau	262	28	234
5	SNPF/PDF Females	Fly 4	12/24 Defau	261	11	250
6	SNPF/PDF Females	Fly 5	12/24 Defau	169	67	102
7	SNPF/PDF Females	Fly 6	12/24 Defau	605	133	472
8	SNPF/PDF Females	Fly 7	12/24 Defau	539	46	493
9	SNPF/PDF Females	Fly 8	12/24 Defau	442	4	438
10	SNPF/PDF Females	Fly 9	12/24 Defau	717	163	554
11	SNPF/PDF Females	Fly 10	12/24 Defau	399	43	356
12	SNPF/PDF Females	Fly 11	12/24 Defau	365	69	296
13	SNPF/PDF Females	Fly 12	12/24 Defau	711	182	529
14	SNPF/PDF Females	Fly 13	12/24 Defau	462	41	421
15	SNPF/PDF Females	Fly 14	12/24 Defau	532	19	513
16	SNPF/PDF Females	Average	12/24 Defau	504.5	97.5714	406.9286
17	SNPF/PDF Females	SEM	12/24 Defau	57.9489	31.6748	36.9458
18	SNPF/PDF Males	Fly 1	12/24 Defau	1430	715	715
19	SNPF/PDF Males	Fly 2	12/24 Defau	363	126	237
20	SNPF/PDF Males	Fly 3	12/24 Defau	1273	607	666

FIGURE 9. SCAMP-generated data spreadsheet. This example shows data for total minutes of sleep, averaged across the days selected by the user for a full 24-h period and each 12-h period. Averages and standard errors of the mean (SEM) are automatically calculated for each group. Similar output spreadsheets are available for most SCAMP analyses.

Specific instructions are provided in Protocol: ***Analysis of Positional Preference in*** **Drosophila** ***Using Multibeam Activity Monitors*** *(Porter et al. 2024) and in Supplemental Document 1 (Vecsey SCAMP_v4 Instructions, https://doi.org/10.6084/m9.figshare.25033670) about how to prepare experiments using these monitors to allow for data analysis using this function. The ability to use the multibeam monitor data to create graphs of fly locations along the tube is an advantage of the version 4 edition of SCAMP (see Fig. 8). These are the current available analysis functions for data from these monitors, but SCAMP will continue to be updated in the future to allow for further graphical and tabular outputs based on four-beam monitor data.*

Excel-compatible .csv export files are available for many graphical outputs, facilitating further statistical analysis using statistics software of your choice, such as JMP or GraphPad (see Fig. 9).

DISCUSSION

Many software options exist to help analyze DAM data; however, some of these involve expensive subscriptions, some primarily examine activity rhythms without allowing for thorough analysis of sleep patterns, some are limited to particular computer systems or are otherwise ungainly to work with, some are not actively supported, and almost all are only equipped to analyze data from DAMs with just a single central beam. SCAMP is freely available, can analyze many parameters of both behavioral rhythms and sleep, can be used on both Macs and PCs, is intuitive to work with, is actively supported, and in its latest version can analyze data from multibeam DAM5Ms as well as single-beam monitors.

More detail about setting up DAM experiments and available methods for the analysis of DAM-based data can be found in Introduction: **Activity Monitoring for Analysis of Sleep in *Drosophila melanogaster*** (Sitaraman et al. 2024) and Protocol: **Neural Stimulation During *Drosophila* Activity Monitor (DAM)-Based Studies of Sleep and Circadian Rhythms in *Drosophila melanogaster*** (Vecsey et al. 2024). Also see Supplemental Document (Vecsey SCAMP_v4 Instructions, https://doi.org/10.6084/m9.figshare.25033670) for a more detailed step-by-step walkthrough for setting up and using the software.

ACKNOWLEDGMENTS

We gratefully acknowledge the following sources of funding: National Institutes of Health (NIH) Academic Research Enrichment Awards (AREA) grants 1R15NS101692-01A1 and 2R15NS101692-02A1 (to C.G.V.), R03NS128513-01 (to G.R.), and College of Science Start-Up Funds (CSUEB), NIH AREA grant 7R15GM125073-02, and National Science Foundation Division of Integrative Organismal Systems (IOS) grant 2042873 (to D.S.). We also thank Martha Brinson at the University of Mississippi for technical assistance.

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Cold Spring Harbor Protocols

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Cold Spring Harb Protoc; doi: 10.1101/pdb.prot108182; published online February 9, 2024