UC Davis MMPC-Live Protocol

Meal Pattern Analysis and Diet/Taste Preference

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Edited by: Michael Goodson - UC Davis Metabolism & Metabolic Health Core

Summary:

Behaviorally, meals are defined as periods of intense feeding and drinking separated by periods of activity, grooming, and rest. A “meal” is the primary data unit for characterizing food intake behavior. Changes in the patterning of meals (number, size or duration, and intermeal interval) may affect food intake and energy balance indirectly. "Meal", in our SOP, is defined by a minimum food intake of 0.02g and at least 10 min between food bout events. Detailed analysis of food intake behavior includes an assessment of average meal duration, average meal size, number of meals, average intermeal interval (IMI), as well as calculation of satiety ratio. In addition, the diurnal (light cycle/dark cycle) patterns of food intake behavior are also calculated to examine temporal shifts in feeding behavior. Meal pattern and food intake behavior is measured in the Research Diets BioDAQ system within bedded home cages that are retrofitted with up to two precision hopper scales/gate controllers (PSCs). The hoppers can be used to measure diet and/or water intake. The cages also have the ability to deliver diet or water using the cage lids depending on what is being measured on the PSCs.

Animals are acclimated to the facility for at least 1 week. Animals are then placed into the “identical” BioDAQ home cages, where they continue to receive pelleted diet and water. Typically at least one week of data are collected and the first set of light/dark cycles are discarded as aclimation to the new cages/hoppers. Because animals are in a home cage environment data can be collected over extended periods of time and for complex experimental designs.

The procedure for diet/taste preference is largely the same as for meal patters, except 1) typically there are two diet or water hoppers on the same cage with the different diets or drinking water options, and 2) the interest is generally in comparing total intake beween the two, though standard meal pattern paramters can also be analyzed from the data as well. It is important in these comparisons, that the side for each diet of drinking water choice be counterbalanced in each group and changed between days if the analysis is carred out over multiple days (typical) to insure there isn’t a cage side bias.

Reagents and Materials:

Reagent/Material	*Vendor*	*Stock Number*
BioDAQ E3 32 PSC/cage Automated Episodic Food & Liquid Intake Monitor for Mice	Research Diets, Inc.	
Conventional water bottles for diet studies		
Diets or water as needed for the study	Columbus Instruments 1-800-669-5011	

Protocol:

Notes on the software:

There are two pieces of BioDAQ software, Monitor and Data Viewer. Monitor is used to acquire the data and interact with the system. Data Viewer is used to view, export data to Excel and do basic analyses of the data. The Data Viewer software is available to be installed on any computer so data analysis does not need to be performed on the BioDAQ computer.

The experimental files consist of five tab-delimitted files, all with the same <root name> followed by an underscore suffix:

- <root name>_settings.tab - *this files records all of the general settings for the system including which PSCs are actively being used*
- <root name>_comment.tab – *this files records when the system notes and anomalous event or the researcher enters a comment*
- <root name>_environment.tab – *this file stores, temperature, humidity and light intensity of in the room/BioDAQ system*
- <root name>_experiment.tab – *this is the main file and the one that you name (except for the suffix). The rest of the files are named based on it. This file contains all of the intake data from the PSCs*
- <root name>_gate-state.tab – *this file contains the state of the gate at any state during the experiment*

To create a new experiment, open the Monitor software. Both the "Central Controller Found" and "Key Valid" indicators should be green and say "Y". If not (unusual), generally restarting the controller, computer and WiFi Router are necessary. Click "Start" on the System Startup screen. When creating an experiment for the fist time, it automatically suggests a name that is a date time code. Generally naming files with the date the recording is started followed by some description of the study and/or study phase is helpful. Also, including the word setup somewhere in the name of the file created when setting up the system can be helpful to indicate that the data contained in this file does not need to be included in the analysis of the experiment.

When "exporting" the data in the middle of the run, click "Stop" on the Recording Data screen in the Monitor software. It is not necessary to fully exit the Monitor software, though there is no harm in doing so. Once, data recording is stopped an the Monitor software is back on the System-Startup screen. Go to Windows File Explorer and copy the data files (see above for the list of all five files) to a USB flash drive. The files are located in C:\BioDAQ\data\. Failing to stop the system before copying files can result in unrecoverable data loss according to the software manual. To restart the recording, click "Start". Instead of creating a new file name, click the file

ending in "_experiment" that you were using when you stopped the recording and it will continue to record data in that file. The controller unit has the capacity to store over a month's worth of data if the computer is offline, so no data will be lost during your export.

The manual also recommends creating new files ever couple of weeks so that the files don't become so large that it causes the Data Viewer software to take a very long time to load and redraw data.

Acclimation and Preparation

1. Single house mice to acclimate them to the cages/bedding/diet in the Meyer Hall vivarium/Room 134.
2. After acclimation, prepare the BioDAQ cages fitted with the appropriate PSCs/blocking plates as needed. For simple diet intake experiments a single PSC for the diet and a blocking plate is all that is required, allowing for monitoring of 32 cages/mice. Water in this case is provided using standard water bottles on the cage tops. For diet or tase preference tests or simultaneous monitoring of diet and water intake, cages are fitted with two PSCs and hoppers for either water or diet, allowing form monitoring of only 16 mice/cages.
3. With the cages/PSCs set up. Start a new set of experimental files in the BioDAQ monitor software.
4. Validate the calibration on each PSC by placing the 10g calibration weight in the empty hopper. Allow the PSC to stabilize. It will recorder the change in mass that it measured. It should be 10.0 ± 0.1g (i.e. 9.9-10.1 g). When adding the weight, the mass will be negative (the system reverses the sign of the weight change so that consuming diet appears to be a positive number). Remove the weight and also note the change in weight. If the measured weights are outside of the 10.0 ± 0.1g range, revalidate the calibration two more times. If both of the next two sets are not within that range, see the BioDAQ manual on how the recalibrate the PSC. After recalibration, revalidate the PSC as before.
5. Once all of the PSCs have been validated, close all of the gates. remove the hoppers and add food and/or water and replace the filled hoppers.

> *Note:* Adding or removing weights >9g does not get logged by the software, so filling/cleaning hoppers does not affect the data. On issue can be the small plastic couplers that are used to attach the hoppers to the PSC weigh much less that that. If they come out, either leave them out and reinsert them when you put the hopper back on the PSC or put the back on before the PSC reads "stable", otherwise the addition of the coupler will get recorded as a negative bout.
>
> ***Note on water hoppers**:* It is possible to put the square BioDAQ water bottles into the hoppers in any of the four directions. Only the one with the sipper tube facing the cage will give the mice access to water (a significant design flaw). We have colored one side of the water bottle green with a Sharpie. When inserted correctly the slit on the back of the hopper (that you can see) should be green. **If it is white, you have put the water bottle in wrong and the mice will have no water**. Please check this each time.

6. Add the mice to the cages.
7. Once all of the PSCs are green and indicate "Quiet". Click stop to end the "Setup" recording.

Data Acquisition

1. Click start and create a new set of experiment files for the actual experiment.
2. Click Open Gate or Resume if using gate scheduling. Verify all gates are open to ensure mice have access to food and water.

3. Check cages daily to ensure mice have diet and water. Depending on the extent of shredding is usually necessary to empty the diet hopper crumb trays every couple of days. To do this:
    a. Wait for all PSCs to indicate "Quiet" and close all gates.
    b. Remove the hopper to be cleaned. Key track of the plastic coupler. If it comes out on the hopper just lay it aside.
    c. Place your hand over the top of the hopper and invert it over a trash can. Shake it to remove crumbs and any bedding the mice may have pushed into the hopper.
    d. Add diet as needed.
    e. Replace hopper (and couple if it came off the PSC).
    f. Repeat for all hoppers that need cleaning.
    g. Add a note that hopper maintenance was done in the notes section.
    h. Once all PSCs indicate "Quiet", reopen all the gates.
4. At the end of the study (or after two weeks) "Stop" the Data Acquisition and copy all of the experimental files to their permanent storage location.

Data Analysis

1. Open the data file (it will ask for the location of the "_experiment.tab" file with Data Viewer. The other files should be in the same location.
2. Set the analysis parameters:
    - Reset Period: "Time of Day", Periods/Day=2, and Experiment Day) start is 12 hours before the change of lights off on the first day. Meyer hall uses mechanical timers so lights on may not be exactly twelve hours before this. Also, the time may not be exactly 6 am (or 7 am depending on daylight saving time). The switch to lights off is the more critical time than the switch to lights on, so we use this value for the 12/12 cycle. To figure out this value, got the "Files" tab and select "Environment" and scroll down until you see the value in the first column after the time of day change from ~24 to ~02 (see screen shot below). In the screenshot, this value is 18:52:06, so we set the start time to 06:52:00.
    - Bouts Filter (1): Max. = 0.40 and Min.= -.02 This excludes bouts that are clearly just shredding or negative bouts that involve the mice pushing bedding into the hopper or urinating in the hopper.
    - Meals Filter: IMI (s) = 600 and Min.=0.02. This defines the minimum size for a meal and the minimum duration between bouts to constitute a new meal.
    - Assign the PSCs to the appropriate group for mice. In this example all mice are in the same group, so they are all "A"
3. Click on Apply.
4. Click on Export .xlsx. After the export finishes and is open in Excel, save the exported file of the data for whatever subsequent analysis is desired. There are tabs that will give you preliminary data from bouts and meals including average bout/meals size, duration, inter-meal interval and total consumption during the light and dark period. **Note: for diet, the consumption with need to be converted to kcal based on the caloric density of each diet before reporting the results.**

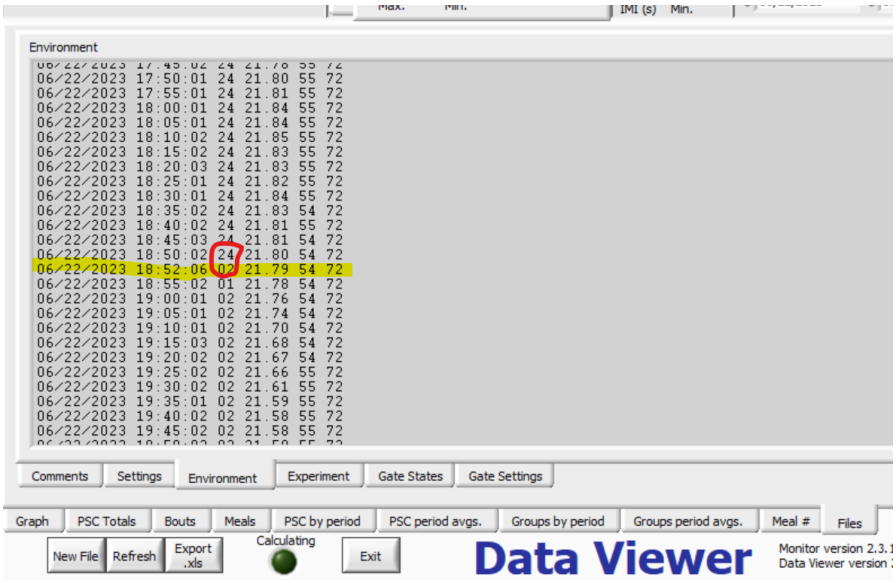

Figure one. Environment File data.

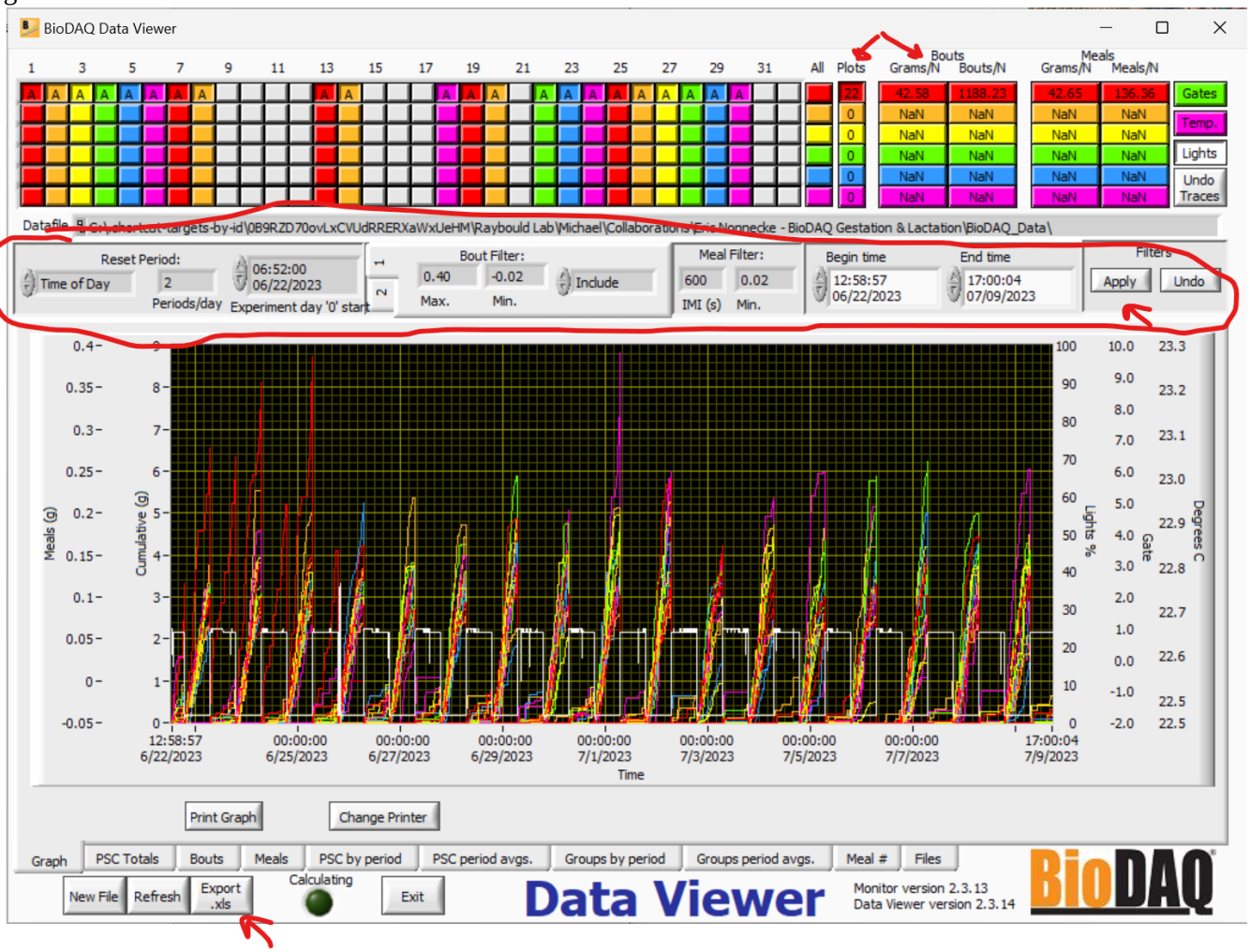

Figure 2. Standard Parameters for intake analysis.

Reagent Preparation:

None.