



PROMETHION  
**Data Analysis Guide**  
2020

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## ExpeData

ExpeData allows the user to explore acquired data in a rich graphic environment where each parameter of the system has been recorded. All data is stored in raw form; therefore, all data analysis is retraceable and will stand up to peer review. Raw data storage also allows for future analyses to be performed on a previous experimental run, ensuring versatility of your data and experimental time.

### Opening and Reviewing Data Files

1. Start ExpeData.
2. Open a data file. Click on File → Open or Ctrl + O. Choose a file, and open it by double-clicking the file name or clicking Open.
3. Review the test contained in the yellow box at the bottom left of the screen. The explanation for each test line is as follows:

good-multiday.exp	File Name
11/11/2013, 09:20:56	Date and time at which the file ended
Channels: 95	Number of data channels in the file
Active: 1 (O2_A)	Actively displayed 'Active' channel
Instrument: Promethion GA3 A	Instrument for 'Active' channel data
Samples: 248488	Number of data samples in the file
1 Sec/sample=69.02 Hrs	Sampling interval & total file duration
Markers: 8246, Status: Original	Number of markers & file status <sup>1</sup>

4. View another data channel by making it the active data channel. Click 'View' → 'Active Channel...' or type Ctrl + A. Choose the data channel you want to view by scrolling through the list, selecting it and clicking 'OK', or by double-clicking on the channel's name.
5. Other data channels can be displayed using Step 4 or keyboard shortcuts. Ctrl + Shift + ← or → can be used to navigate through the data channels with the same channel prefix. Ctrl + ← or → can be used to navigate to the previous or next data channel, respectively.
6. Zoom into the data recording to view it in more detail. Use the mouse wheel to zoom in and out. The blue bar below the graph shows the size and location of the zoomed-in section with respect to the entire recording. View the exact time and date of any point by moving the cursor over the graph while holding down the Ctrl key.

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<sup>1</sup> Files that not have been modified since the file's opening will have an 'Original' status. Any file modification will change the file status to 'MODIFIED'.

## MacroInterpreter

To use the macro interpreter program, simply click on File → Open, select the file and click Open. A list of the channels in the file will appear on the right window, while file details and file remarks will appear in the left window.

If you would like to examine the file in graphical form, click on View → Graph and follow the prompts. Note that you can use the mouse wheel to zoom in and out of the recording as desired. After you have finished examining the file, you will need to close the graphic window.

If you would like a quick statistical summary of the file, click on Spreadsheet → Channel Data to Spreadsheet. The spreadsheet tab on the window will be automatically selected, and the spreadsheet will be displayed. The name of each channel, its position in the file, and its mean, minimum, maximum and range will be displayed.

## Split File

Split File is a MacroInterpreter feature that allows large data files to be split into smaller data files that can be opened in ExpeData. Data files can be split to groupings of cages 1-8 and 9-16 or individual cages. Only the raw data file(s) should be split using the Split File option. Splitting files modified by macros or previously split using this feature may result in incorrect data analysis.

To split a data file, open the MacroInterpreter program. Click File → Open and load the raw data file that needs to be split. Once the file has loaded, click Tools → Split File into 1-8, 9-16 or Tools → Split File to Individual Cages. The file split is complete when Done is printed in the lower left corner of the program window. The smaller, split files are saved to the same directory as the original larger raw data file. Split the files to individual cage if the cage 1-8, 9-16 files are still too large to open in ExpeData.

## Running a Macro

1. Open MacroInterpreter.
2. Load the .exp data file. Click on File → Open, select the raw data file and click Open.
3. Wait for MacroInterpreter to load the raw data file.
4. Select the One-click macro you want to apply. Click on Macros → Load Macro Collection, select the macro and click Open. The analysis tab will be automatically opened, and the text of the macro will be displayed in the right-hand window.
5. To run the macro, click Run. A text window will appear that will display all of the remarks in the macro text. Most macros are abundantly annotated with remarks, which always begin with an apostrophe. Each remark is displayed as it is encountered.
6. Wait for the data analysis to be completed. When the macro is completed, the text window will disappear, and the note Completed will appear in the bottom left of the program window.
7. View the data. After completion, a .xml file is generated in the same .exp raw data directory. Open the .xml via Excel.

In general, MacroInterpreter will be used with comprehensive, one-click macros. In this case, the macro number in the loaded macro collection will be one. If more specialized macros are used, there may be several macro numbers available.

In the case of one-click macros, the file that is automatically produced is in the .xml format. These files can be loaded into practically any spreadsheet program, including open versions. Writing in this format

avoids having to deal with the necessity of having Microsoft Excel loaded on the computer, and also avoids bugs in the Microsoft.net framework that can intermittently occur when transferring data directly to Excel. If you are using Excel, simply load the XML file (usually, these files are associated with Excel so you can simply double-click on the file to load it). After doing so, it is recommended to save the spreadsheet as a .xlsx file, which is approximately 10 times more compact. At that point you can delete the XML file if you wish.

Note that in order to create a useful link between the data file and the macro that created the spreadsheet, the default name for the spreadsheet is created by appending the macro name to the filename. The actual macro number that was called (usually macro 1 for a 1-click macro collection) is appended to this as well, preceded by an underline. For example, if the original data file is named "Test\_Run", and it is analyzed using One-Click Macro V2.38-slice1hr, then the XML file generated by the macro will have the file name of Test\_Run\_macro\_One-Click Macro-slice1hr.mac\_1.

## One-Click Macro Analyses

The intention of the one-click macros is to provide all of the commonly used analytical outputs without requiring the user to sequentially run a variety of macros in a collection. It may contain more data than most users will typically use, but it is hoped that user curiosity will lead to useful information being discovered in the less frequently used options.

A minor edit to the one-click macro may be required for the circadian analysis. If a light level channel does not exist in the data file, you may need to edit the cycle information. To do so, load the macro collection into a text editor (Notepad will do; Notepad++ is a more advanced open source version that is highly recommended). Search for the command “assign circadian\_markers” and edit the diurnal hours duration to the correct number of hours (usually 12), and the diurnal StartTime, using a standard 24-hour format (hh:mm:ss). Save the macro file under a different name that will identify the circadian timing used.

Various analyses will appear in the spreadsheet, separated by worksheets that are accessed in the tabs at the bottom of the program window. These are:

- Time Series
- Macro 13
- Circadian
- Food Intake Pattern
- Wheel Pattern
- Time Budget percent
- Locomotion Budget percent
- Locomotion Budget in meters per hour
- Behavior Transitions
- QC checks
- Metadata

QC checks and Metadata are two housekeeping worksheets. QC Checks performs some basic checks on data integrity. This can pick up many potential issues and should always be checked. Metadata summarizes the important details about the file, including its location, the setup file that was used to specify recording parameters, the date and time the recording stopped, the number of samples, the version of the program acquiring the data, and a wide variety of calibration information for all of the important sensors and transducers in the system. If specific details concerning the animals are entered into the remarks, they can be accessed by the macros (for example, this happens in the circadian data worksheet). In addition, at the bottom of the worksheet, it will show the macro version that was used to create the spreadsheet, and the exact folder in which the macro version is located. The rest of the data analyses will be described in more detail in the following sections.

## Time Series and Macro 13

The Time Series analysis outputs commonly used metrics, with the cage number denoted by the animal column. Thus, in a statistics program, it is straightforward to isolate individual mice or compare groups of mice by using the animal datum to identify them. Macro 13 outputs the same data as Time Series, but with a separate column for each animal. In this case, the animal number is denoted by a numeric suffix preceded by an underline for each column header. *In both worksheets, the units for VO<sub>2</sub> and VCO<sub>2</sub> are mL/minute, units for EE are kcal/hour, units for VH<sub>2</sub>O are grams /hour, units for intake and body mass are grams, units for distance are meters, and units for speed are meters/second.*

### Suffix Definitions

**\_M** This is the mean value of the data obtained during the time that metabolic data were obtained from the cage. Typically, in Promethion systems, the metabolic data are obtained in 30 seconds for each cage. By default, a baseline (which measures the incurrent values of oxygen, carbon dioxide and water vapor) is taken every four cages. Each analyzer chain in a Promethion system is assigned eight cages (typical Promethion systems contain one, two, or three analyzer chains). Thus, individual samples are taken from each cage every five minutes, irrespective of the size of the complete system. (This can be reduced every 2.5 minutes with some careful tuning of the system and can also be reduced by assigning fewer than eight cages to each analyzer.) Data on VO<sub>2</sub>, VCO<sub>2</sub>, VH<sub>2</sub>O, energy expenditure (kcal/hr) and RQ are best obtained at the moment of cage sampling. Certain other forms of data which are calculated in cumulative fashion, such as food and water intake and body mass, pedestrian meters run and wheel meters run etc., can also be measured during this time and will also be given the \_M suffix. But for certain other types of data, it does not make sense to examine only the data during the time when the gas sample from the cage was being analyzed.

**\_R** This is the range of the data across an entire cycle of measurement (R=Range), in other words, from the end of the previous time the cage was selected until the end of the current cage measurement. By default, in Promethion systems, this is every five minutes. If we are interested (for example) in the distance a mouse traveled by foot during each measurement interval, it would make no sense to look at the data range within only the period of metabolic measurement, which is typically just 30 seconds long. Rather, we are interested instead in the total distance the mouse traveled since the cage was last selected.

**\_MNZ** This is the mean of the data across the entire cycle of measurement (M=Mean) but with zero values excluded from the mean calculation (NZ=No Zeros). This is useful for extracting actual pedestrian mall wheel running speeds. For much of the time, the mouse will not be moving or running at all and thus the speed during those times will register as zero. This will tend to drag down the mean speeds by an unpredictable amount, depending on the amount of time for which the mouse was not moving. To avoid this, excluding zeros from the calculation gives a more faithful data extraction that reflects the actual speeds at which the mouse typically moved. Recall that the total distance moved is available as another column, so you do not lose information on the actual distance the mouse moved.

**\_X** Denotes the cage number for animal X

## Column Prefix & Title Definitions

**AllMeters** Sum of all distances traveled within the beam break system, not including any distance run on the wheel (WheelMeters), in meters (m). This includes fine movement (such as grooming and scratching) as well as direct locomotion. AllMeters are always greater than PedMeters. To get an index of fine activity, subtract PedMeters from AllMeters.

**Animal** Cage number.

**BodyMass** Mean body mass of animal, in grams (g).

**BodyTemp** Mean body temperature of animal in cage X, in degrees Celsius (°C). Telemetry is required to acquire this data.

**FoodInA** Mass of food consumed by animal during the cycle, in grams (g).

**FoodInB** Mass of food consumed by animal during the cycle, in grams (g). This data will only exist if there is a second food option present.

**HeartAmp** Heart rate amplitude, in beats per minute (BPM). Telemetry is required to acquire this data.

**HeartRate** Heart rate, in beats per minute (BPM). Telemetry is required to acquire this data.

**kcal\_hr** Mean energy expenditure (EE) of animal, in kilocalorie per hour (kcal/hr). EE is calculated using the Weir equation.<sup>2</sup>

**PedMeters** Sum of all directed ambulatory locomotion within the beam break system, excluding all wheel locomotion, in meters (m). For defining directed locomotion, the macros use a speed cut off of 1 cm/s. If the animals travel at that speed or faster, it is considered to be directed locomotion.

**PedSpeed** Mean directed ambulatory locomotion speed, in meters per second (m/s).

**RER** Mean respiratory exchange ratio (RER),  $VCO_2/VO_2$ , unit-less.

**TelAct** Summation of activity. The Gross Motor Activity unit of measure is a Count. A Count represents the frequency and intensity of the subject's movement per second. Depending on what sampling output is selected, all activity generated per second is stored in a buffer then outputted in a total count output at the conclusion of your selected sample size. Telemetry is required to acquire this data.

**TreadAngle** The angle of the treadmill in degrees.

**TreadSpeed** The speed of treadmill given in meters per minute (m/min).

**TreadShocks** The number of times the animal was shocked while on the treadmill.

**VCO2** Rate of carbon dioxide emission ( $VCO_2$ ), in milliliters per minute (ml/min).

**VH2O** Rate of water vapor loss ( $VH_2O$ ), in milliliters per minute (ml/min).

**VO2** Rate of oxygen consumption ( $VO_2$ ), in milliliters per minute (ml/min).

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<sup>2</sup> Weir (1949): New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol. 109:1-9.



**WaterInA** Mass of water consumed by animal during the cycle, in grams (g).

**WheelMeters** The number of meters (m) run on the wheel, not including any ordinary pedestrian locomotion around the cage.

**WheelSpeed** Mean wheel speed when animal is running on wheel, in meters per second (m/s).

**XBreak** Number of X-axis beam breaks.

**YBreak** Number of Y-axis beam breaks.

**ZBreak** Number of Z-axis beam breaks.

## Circadian

The extracted data from the Circadian analysis are separated by circadian cycle, denoted in the Cycle column (0 = nocturnal, one = diurnal). If data on the age, genotype, ID, sex or initial mass of the animal are recorded in the file remarks, these are extracted into the appropriate columns. All column names refer to the measured variable across the entire duration of the circadian cycle. Note that this analysis includes food intake pattern analysis.

### Column Prefix & Title Definitions

**A\_EE\_15** Mean energy expenditure (EE) during the 15 minutes with highest EE, in kilocalorie per hour (kcal/hr). EE is calculated using the Weir equation.<sup>3</sup>

**A\_RER\_15** Mean respiratory exchange ratio (RER) during the 15 minutes with highest energy expenditure (EE), unit-less.

**AccessA\_pct** Sum of the time that the animal has unrestricted access to the food, as a percentage of cycle (%).

**Adur\_hrs** Sum of animal's time active during the highest activity 15-minute period, in hours.

**AllMeters** Sum of all distances traveled within the beam break system, not including any distance run on the wheel (WheelMeters), in meters (m). This includes fine movement (such as grooming and scratching) as well as direct locomotion. AllMeters are always greater than PedMeters. To get an index of fine activity, subtract PedMeters from AllMeters.

**Animal** Cage number.

**Age\_months** Animal's age, in months. Extracted from user-entered information in Animal Data Entry.

**Avg\_EE** Mean energy expenditure (EE), in kilocalorie per hour (kcal/hr). EE is calculated using the Weir equation.<sup>3</sup>

**Avg\_RER** Mean respiratory exchange ratio (RER), unit-less.

**Avg\_VCO2** Mean rate of carbon dioxide emission (VCO<sub>2</sub>), in milliliters per minute (ml/min).

**Avg\_VH2O** Mean rate of water vapor loss (VH<sub>2</sub>O), in milliliters per minute (ml/min).

**Avg\_VO2** Mean rate of oxygen consumption (VO<sub>2</sub>), in milliliters per minute (ml/min).

**BodyMass\_g** Mean body mass of animal, in grams (g).

**BodyMass\_SD** Standard deviation of body mass measurement, in grams (g).

**BP\_kPa** Mean barometric pressure, in kilopascal (kPa).

**Cycle** Circadian cycle state. 1 is nocturnal, 0 is diurnal.

**Cycle\_Hrs** Length of cycle, in hours.

**Day** Number of days from the start of the run.

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<sup>3</sup> Weir (1949): New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol. 109:1-9.

**EndTime** Cycle end time.

**Genotype** Animal's genotype. Extracted from user-entered information in Animal Data Entry.

**Id\_code** Animal's identification code. Extracted from user-entered information in Animal Data Entry.

**InitMass\_g** Animal's initial mass, in grams (g). Extracted from user-entered information in Animal Data Entry.

**InterUptakeA\_min** Mean food inter-intake event duration, in minutes. This data is from the first food hopper present.

**InterUptakeB\_min** Mean food inter-intake event duration, in minutes. This data will only exist if there is a second food hopper present.

**InterUptakeW\_min** Mean water inter-intake event duration, in minutes.

**PedMeters** Sum of all directed ambulatory locomotion within the beam break system, excluding all wheel locomotion, in meters (m). For defining directed locomotion, the macros use a speed cut off of 1 cm/s. If the animals travel at that speed or faster, it is considered to be directed locomotion.

**PedSpeed** Mean directed ambulatory locomotion speed, in meters per second (m/s).

**QA\_EE\_15** Mean energy expenditure (EE) during the highest EE 15-minute "quiet" period, in kilocalorie per hour (kcal/hr). EE is calculated using the Weir equation.<sup>4</sup>

**QA\_RER\_15** Mean respiratory exchange ratio (RER) during the highest energy expenditure 15-minute "quiet" period, unit-less. "Quiet" is defined as time in which the animal was not engaged in eating, drinking, grooming, nor locomotion.

**QR\_EE\_30** Mean energy expenditure (EE) during the lowest activity 30-minute period, in kilocalorie per hour (kcal/hr). This would be the approximation of resting metabolic rate because it is for the period when the animal "rested" the longest. EE is calculated using the Weir equation.<sup>4</sup>

**QR\_RER\_30** Mean respiratory exchange ratio (RER) during the lowest activity 30-minute period, unit-less.

**R\_EE\_30** Mean energy expenditure (EE) during the lowest EE 30-minute period, in kilocalorie per hour (kcal/hr). EE is calculated using the Weir equation.<sup>4</sup>

**R\_RER\_30** Mean respiratory exchange ratio (RER) during the lowest energy expenditure (EE) 30-minute period, unit-less.

**Rdur\_hrs** Sum of animal's time resting during the lowest activity 30-minute period, which is defined as no ambulatory nor wheel locomotion, in hours.

**Run\_pct** Sum of animal's time spent running on wheel, as a percentage of cycle (%).

**Sex** Animal's sex. Extracted from user-entered information in Animal Data Entry.

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<sup>4</sup> Weir (1949): New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol. 109:1-9.

**Sleep\_hrs** Sum of animal's sleep, in hours. The animal is considered sleeping when the animal has been "quiet" for more than 40 seconds.<sup>5</sup> Animal's "quiet" time is defined as time in which the animal was not engaged in eating, drinking, grooming, nor locomotion.

**Sleep\_pct** Sum of animal's sleep, as a percentage of cycle (%). The animal is considered sleeping when the animal has been "quiet" for more than 40 seconds.<sup>5</sup> Animal's "quiet" time is defined as time in which the animal was not engaged in eating, drinking, grooming, nor locomotion.

**StartDate** Cycle start date.

**Still\_pct** Animal's "quiet" time, which is the time at which the animal was not engaged in eating, drinking, grooming, nor locomotion, as a percentage of cycle (%). Still\_pct is always greater than Sleep\_pct. Subtract Sleep\_pct from Still\_pct to get the time the animal is "quiet," but not asleep.

**StartTime** Cycle start time.

**Tot\_EE** Sum of energy expenditure (EE) in kilocalories. EE is calculated using the Weir equation.<sup>6</sup>

**UptakeA\_g** Mean mass of food consumed during an intake event, in grams (g).

**UptakeA\_min** Mean food intake event duration, in minutes.

**UptakeA\_N** Number of food intake events.

**UptakeA\_Sum** Sum of all food consumed, in grams (g).

**UptakeB\_g** Mean mass of food consumed during an intake event, in grams (g). This data will only exist if there is a second food option present.

**UptakeB\_min** Mean food intake event duration, in minutes. This data will only exist if there is a second food option present.

**UptakeB\_N** Number of food intake events. This data will only exist if there is a second food option present.

**UptakeB\_sum** Sum of all food consumed, in grams (g). This data will only exist if there is a second food option present.

**UptakeW\_g** Mean mass of water consumed during an intake event, in grams (g).

**UptakeW\_min** Mean water intake event duration, in minutes.

**UptakeW\_N** Number of water intake events.

**UptakeW\_Sum** Sum of all water consumed, in grams (g).

**Walk\_pct** Sum of animal's time spent in ambulatory locomotion, as a percentage of cycle (%).

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<sup>5</sup> Pack, A, Galante, R, Maislin, G et al. (2007): Novel method for high-throughput phenotyping of sleep in mice. *Physiological Genomics* 28, 232-238.

<sup>6</sup> Weir (1949): New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol.* 109:1-9.

**WheelMeters** The number of meters (m) run on the wheel, not including any ordinary pedestrian locomotion around the cage.

**WheelSpeed** Mean wheel speed when animal is running on wheel, in meters per second (m/s).

**XBreaks** Number of X-axis beam breaks.

**YBreaks** Number of Y-axis beam breaks.

**ZBreaks** Number of Z-axis beam breaks.

## Food Intake Pattern

The Food Intake Pattern Analysis will output detailed information for every intake event for each animal during the entire experiment. An intake event is detected by scanning the food hopper mass channel for the high standard deviation in the measured mass caused by the animal interacting with the hopper. Internally, the algorithm creates a standard deviation vector of the mass channel, with a sliding window size that is set in the "SD slide window" box. When the standard deviation rises above the value in the "Standard deviation threshold for intake" box, the algorithm suspects that a feeding event is taking place. If the threshold value is set too low, the intrinsic noise in the measurements will continually give false triggers. In general, the minimum standard deviation threshold is set to 0.02 g for water intake, and 0.05 g for food intake. This difference is because water intake is a kinder, gentler process than food intake, resulting in a smaller standard deviation signal. The different parameters that are used to detect food and water intake events are listed in Table 1 below.

Table 1: Food and Water Intake Parameters

	Minimum Intake Duration (Seconds)	Maximum Pause Seconds	Standard Deviation Threshold	Maximum Probability	Minimum Uptake Mass (g)	Maximum Uptake Mass (g)
Food	30	150	.05	.05	0	1
Water	10	30	.02	.05	0	1

After a suspected intake event is detected, the algorithm waits until the standard deviation drops below the threshold. Of course, momentary pauses in food intake may occur, and it is important that the algorithm is not fooled into thinking that the entire intake event is over. As a result, a value for the "maximum inter-intake pause duration" is obtained from the appropriate box in the dialog. This ensures that brief pauses in intakes are not considered to be the end of an intake event. By default, the maximum inter-intake pause duration is set at 300 seconds. It can be set lower if detailed intake pattern analyses are needed, which require more granular data.

Similarly, the value in the "Minimum intake duration" box determines the minimum duration of an intake event. This is particularly useful for water intake events, which generally last about 5-30 seconds. Thus, the minimum intake duration should be set to 10 seconds for water intake. For food intake, which generally takes longer, 30 seconds is usually appropriate. Note that with water intake analysis, dripping from the sipper tube (which can take place during sudden temperature changes) causes a sharp, momentary drop in the water hopper mass that will be rejected as a valid reading.

When an intake event is detected, and it lasts for at least as long as the minimum event duration (in seconds), and is separated from the previous intake event by at least the maximum inter-intake pause duration, the algorithm marks the beginning and the end of the intake event by finding at least six consecutive samples of the standard deviation vector that are below the standard deviation threshold. The search is made downstream (in the past) in the case of intake initiation, and upstream in the case of intake termination.

The algorithm next determines the mean and standard deviation of the most level section of eight consecutive samples 60 seconds prior to, and 60 seconds after, the intake event. The two means and standard deviations are then plugged into a two-sample, equal N Student's t-test, and the resulting T statistic is evaluated for the probability that the two means are equal. By default, a valid intake event should have a probability of less than or equal to 0.05. This probability can be set in the "maximum probability for uptake" event box. If an intake event evaluates to a probability above the maximum, it is ignored.

Because mice will occasionally drop pieces of bedding and other objects into the food hopper, which will result in a false negative mass reading, the system by default ignores intakes below the value in the "signal intakes less than this mass" box. Such readings can also be caused by freak interactions between the mouse and the hopper. Likewise, such interactions can cause spurious positive intake events that can be quite large, especially when food access is unblocked owing to the mechanical interaction between the food access control door and the food hopper. For this reason, intakes above the value in the "signal intakes greater than this mass" box are ignored.

Optionally, "meals" can be analyzed in the case of intake pattern analysis. Meals are regarded as groups of intake events separated by longer pauses than individual intake events; the default maximum pause duration is set in the "maximum pause duration within meal" box. This setting is only valid for intake pattern analysis.

If intake processing occurs without a selected data section (in other words, for the entire duration of the recording), the default mode of action is to calculate cumulative intake, and the cumulative total intake replaces the original data. If a data section is selected, detailed intake pattern analysis occurs by default. For example, the circadian macro selects day and night sections of the intake channels and performs detailed intake pattern analysis on them, whereas the standard "macro 13" output first transforms the intake channels into cumulative totals, and then calculates the range for each output point.

Detailed intake pattern analysis can optionally be saved to the clipboard or to a file in the folder of the original data file.

It is easy to create macros that cycle through different values of the key parameters by using a for/next loop. If the resulting values are written to a spreadsheet, it is possible to visualize the effect of the parameters on (for example) total intake amounts.

#### Column Prefix & Title Definitions

**Accum\_g** The total cumulative amount of food eaten, in grams (g).

**Cage** Animal number

**EndDate** The date at which the current intake event ended

**EndTime** The time at which the current intake event ended

**Event** Food intake event number

**In\_g** The amount of food eaten during the current intake event, in grams (g).

**In\_g\_min** The intake rate during the current intake event, in grams per minute (g/min).

**In\_min** The duration of the current intake event, in minutes.

**InF\_g\_min** The amount of force that the mouse exerted on the hopper, obtained by integrating under the mass profile during the intake event, in grams per minute (g/min).

**InterIn\_min** The inter-intake interval immediately prior to the current intake event, in minutes. For the 1st intake event, this inter-intake interval is unknown, thus it is indicated as 0.

**Prob** The probability that the food intake event was the result of chance ("false positive.")

**StartDate** The date at which the current intake event started

**StartTime** The time at which the current intake event started

**Stud\_t** The Student's t-statistic calculated from the mass and standard deviation of the mass of the hopper immediately prior to, and immediately after the end of the feeding event. The t statistic is then used to calculate the probability, given the actual mass measurement precision found in the file, that the food intake event was the result of chance. By default, this probability is set to  $< 0.05$ . The vast majority of intake events are below .001 probability, which is indicated by 0.



## Wheel Pattern

Similar to the food intake pattern analysis, this analysis will output detailed information for each wheel running event during the entire experiment for each animal.

As you know, mice do not run continuously on the running wheel. They will occasionally stop for a second or two or three, and then continue running again. The wheel pattern analysis assumes that if a pause (i.e. the mouse being still) lasts for longer than five seconds, any subsequent running should be counted as a separate running event. The value can be changed by modifying the “max\_pause” parameter in the macro.

This means that in some running bouts, the mouse may have paused for a few seconds, with no pause lasting longer than five seconds in the case of the current macro parameters. The "still" column shows the total number of seconds during which the mouse did not run on the wheel during the running bout.

Because the actual duration of wheel-running can be different from the duration of the complete running bout (the format excludes the still periods, while the latter includes them), the running speed of the mouse on the wheel is best measured by the distance it runs divided by the actual duration of wheel running, not including the still periods. This is the column RunMeters\_sec. However, if for some reason you want to know the average speed of the running during the entire wheel running bout including the still periods, this is available in the column AvgMeters\_sec. Thus, RunMeters\_sec will always be higher than AvgMeters\_sec unless the mouse had absolutely no pauses during the running bout.

## Column Prefix and Title Definitions

**Animal** Cage number.

**AvgMeters\_sec** The average running speed during the entire wheel running event measured by the total distance run divided by the total time of the event including the still periods, in meters per second (m/s).

**Dur\_secs** Duration of the current wheel running event, in seconds.

**EndTime** The time at which the current wheel running event ended

**Joules\_area\_hrs** Used to estimate net cost of transport by regressing meters run per running bout (x) against Joules of net energy expended during each bout. (Joules is labeled Joules\_area\_hr because it is obtained by integrating the area under the curve of Joules per hour against time in hours). The slope of the line is the NCOT in net Joules per meter.

**PostRun\_secs** The time interval between when the current running wheel event ends and the next wheel running event starts, in seconds.

**PrevRun\_secs** The time interval between when the previous running wheel event ends and the current wheel running event starts, in seconds. For the 1st event, this interval is unknown, thus it is indicated as 0.

**RunMeters\_sec** The average running speed during the wheel running event measured by the total distance run divided by the actual duration of wheel running, not including the still periods, in meters per second (m/s).

**Sample** The sample number at which the current wheel running event started

**StartDate** The date at which the current wheel running event started

**StartTime** The time at which the current wheel running event started

**Still\_secs** The amount of time during the current running wheel event that the animal paused on the wheel and was not running, in seconds.

**WheelMeters** The number of meters (m) run on the wheel during the current wheel running event

**Wheel\_Revs** The number of wheel revolutions for the current running wheel event.

## Ethoscan

The Promethion system acquires a uniquely rich stream of data and knows exactly where the animal is, and what it is doing, in any given second. Ethoscan is a tool to examine an animal's behavior and activity and generates a list of each successive behavior. The list of currently available behaviors for analysis are defined below.

Behaviors can be analyzed by monitoring the animal's activity and interactions with cage sensors. For example, if the mass measured by the body mass sensor suddenly increases, it is known that the animal has entered the body mass habitat. If the variance of the food intake monitor measurements suddenly increase, it is known that the animal is interacting with the food hopper. From these interactions, and sometimes even from the lack of such interactions, a detailed sequence of the behaviors exhibited by the experimental animal in each cage can be obtained. If the animal is not actually interacting with any of the internal cage sensors— the running wheel, food hopper, water dispenser, or body mass habitat— then the animal is considered to be “lounging.” Ethoscan analysis distinguishes between a short lounge duration, a lounge between 5 and 60 seconds, and a long lounge, which is simply a lounge lasting longer than a short lounge.

### Behavior Definitions

**EfodA** Eating food from food hopper A, as evidenced by a significant difference in the food hopper mass from before to after the eating event.<sup>7</sup>

**TfodA** Touching food hopper A without actually removing a significant amount of food.<sup>7</sup>

**DWatr** Drinking water, as evidenced by a significant difference in the water dispenser mass from before to after the drinking event.<sup>7</sup>

**TWatr** Touching the water dispenser without actually removing a significant amount of water.<sup>7</sup>

**Wheel** Completing at least one revolution of the running wheel.

**IHome** Entering the body mass habitat for a long enough time to obtain a stable mass reading.

**THome** Touching the body mass habitat or entering very briefly, in such a way that its mass reading disturbed but a stable mass is not available.

**LLnge** Long lounge: wandering around the cage, without interacting with the food or water mass monitors, the body mass monitor, or the wheel, for more than 60 seconds.

**SLnge** Short lounge: wandering around the cage, without interacting with the food or water mass monitors, the body mass monitor, or the wheel, for 5-60 seconds.

**EfodB** Eating food from food hopper B, as evidenced by a significant difference in the food hopper mass from before to after the eating event.<sup>7</sup> This behavior will only appear in the Ethoscan analysis if a second food hopper is present.

**TfodB** Touching food hopper B without actually removing a significant amount of food.<sup>7</sup> This behavior will only appear in the Ethoscan analysis if a second food hopper is present.

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<sup>7</sup> Events are evaluated for statistical significance with a T-test of a 20s baseline sample before vs. after the event.

The behavior list is available across any selected section of the recording for each animal. In addition to the behavior code, each entry in the list contains the duration of the activity, the exact start and stop calendar times, the mean position of the animal in XY coordinates, and a specific quantity associated with that activity. For example, in the case of a long lounge or short lounge, the distance moved is the specific quantity; in the case of food intake, the amount of food eaten; in the case of body mass habitat entry, the body mass of the animal; and so on.

Table 2: Example Ethoscan behavior list.

Sample	Start Date	Start Time	End Time	Duration (sec)	Activity	Amount	Rear (%)	X (cm)	Y (cm)	S (cm)
1	11/8/2013	12:19:30	13:16:25	3416	llnge	22	5.3	8.3	19.7	22
3417	11/8/2013	13:16:26	13:42:17	1552	ihome	22.207	0	10.8	9	0
4969	11/8/2013	13:42:18	13:42:48	31	slnge	21	32.3	6.7	20	21
5000	11/8/2013	13:42:49	13:44:10	82	efoda	0.038	46.3	10.2	20.9	1
5082	11/8/2013	13:44:11	13:44:52	42	slnge	2	7.1	10.3	20	2
5124	11/8/2013	13:44:53	13:48:19	207	efoda	0.044	44	8.8	21.4	52
5331	11/8/2013	13:48:20	13:48:25	6	slnge	3	16.7	7.5	24.6	3
5337	11/8/2013	13:48:26	15:16:44	5299	ihome	22.139	0	7.5	22.8	0
10636	11/8/2013	15:16:45	15:17:22	38	slnge	14	31.6	8.1	24.4	14
10674	11/8/2013	15:17:23	15:56:38	2356	ihome	21.711	0	8.5	21	0

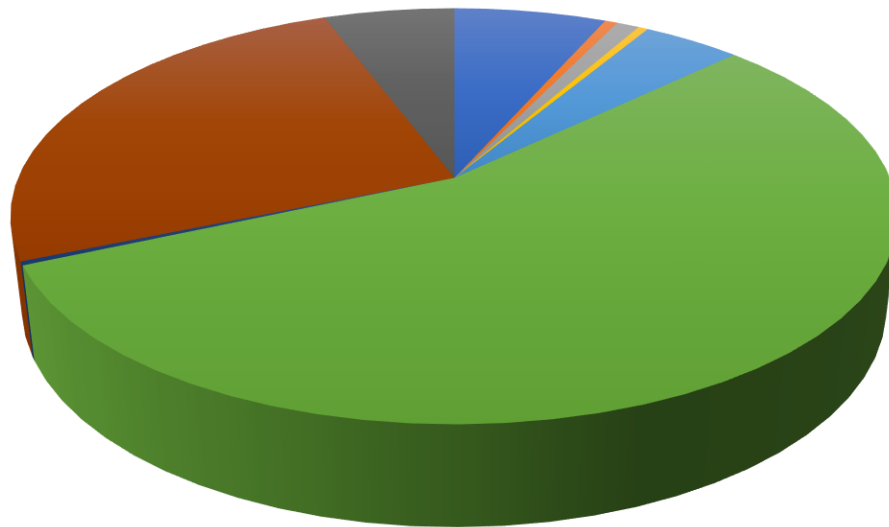
Amount is centimeters (cm) for slnge and llnge, revolutions for wheel, grams (g) for efoda, efodb, and ihome, and milliliters (mL) for dwatr. Rear (%) is the percentage of the behavior duration spent rearing, which is determined from the Z-axis beambreak array. X and Y are the average X and Y beambreak array position during the behavior, respectively. S is the total distance travelled during the behavior.

A lot of information can be derived from the behavior list. The following sections provide example behavioral analyses that are already built into Ethoscan. These analyses give a great deal of information that can be subjected to standard statistical analyses, providing researchers the ability to investigate and quantify differences between control and experimental groups of animals (assuming there is a difference). Treatment effects for injections or cannular transfusions can also be quantified.

## Time Budget

A complete time budget is available for each animal. Each activity is shown in terms of the percentage of the animal's total time engaged in this activity during the selected cycle.

### Time Budget



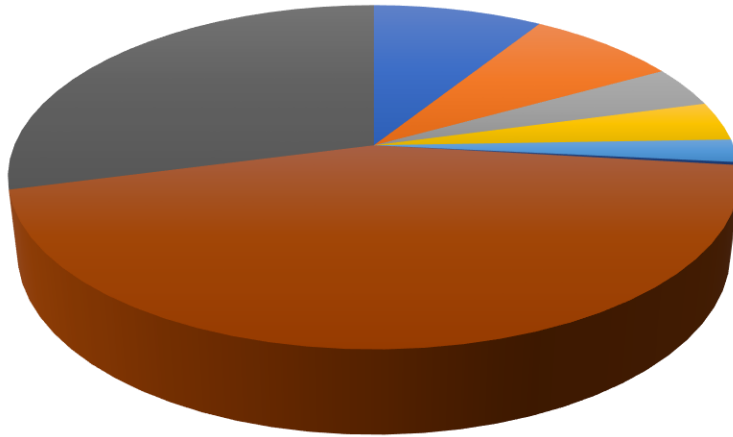
■ EfodA  
 ■ TfodA  
 ■ DWatr  
 ■ TWatr  
 ■ Wheel  
 ■ IHome  
■ THome  
 ■ LLnge  
 ■ SLnge  
 ■ EfodB  
 ■ TfodB

*Figure 1: An animal's time budget*

## Locomotion Budget

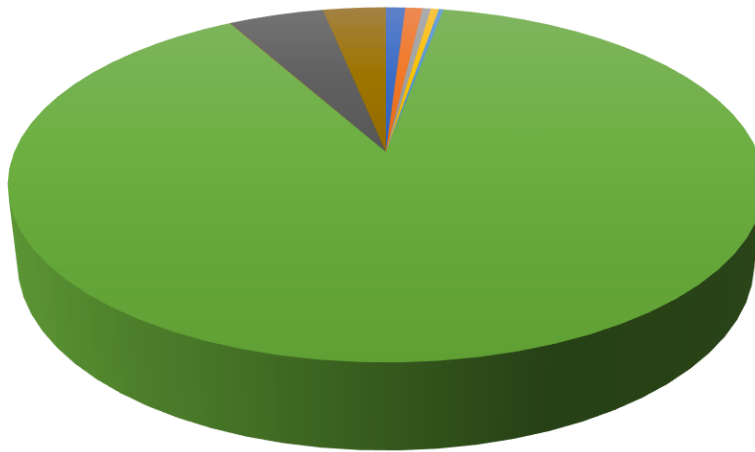
A locomotion budget is available for each animal. This shows the percentage of overall total distance traveled during each of the behaviors recognized by the system. Absolute distances traveled (in meters) are also available for all behaviors.

### Non-wheel Locomotion Budget



■ Animal ■ EfodA ■ TfodA ■ DWatr ■ TWatr ■ IHome ■ THome ■ LLnge ■ SLnge

### With-wheel Locomotion Budget



■ Animal ■ EfodA ■ TfodA ■ DWatr ■ TWatr ■ Wheel ■ IHome ■ THome ■ LLnge ■ SLnge

Figure 2: An animal's non-wheel and with-wheel locomotion budgets.

### Behavior Transition Probability Matrix

A behavior transition probability matrix is available for each animal. Although the term may sound a bit daunting, the concept is simple. Imagine that the animal is eating food. That is only one of several behaviors recognized by the system. When the animal stops eating, what will its next behavior be? The behavior transition probability matrix allows you to determine that easily. For example, 30% of the time, after eating the animal may drink; it may go directly to the running wheel only 2% of the time, to its body mass habitat 40% of the time, and so on. For every behavior, the percentage probability of each possible next behavior is calculated and displayed. In essence, the behavior transition probability matrix tells the researcher, as far as it can, how the mind of the experimental animal works. Moreover, it is objective. It can also be subjected to some interesting and powerful statistical analyses, such as hierarchical object clustering, which in turn allows a dendrogram of animals grouped by the relatedness of their behaviors to be displayed.

Table 3: Example Ethoscan behavior transition probability matrix.

	efoda	tfoda	dwater	twatr	wheel	ihome	thome	llnge	slnge	efodb	tfodb	Sum (%)
efoda	0	0	30.05	0	2.12	40.03	0	12.56	15.24	0	0	100
tfoda	0	0	0	0	7.23	0	0	14.46	78.31	0	0	100
twatr	4.55	0	0	0	0	9.09	13.64	4.55	68.18	0	0	100
wheel	0	1.35	0	0	0	0	1.62	12.94	84.1	0	0	100
ihome	0	0	0	0.67	2.68	0	0	17.45	79.19	0	0	100
thome	0	0	1.75	1.75	5.26	0	0	19.3	71.93	0	0	100
llnge	16.46	12.66	7.59	2.53	25.95	19.62	15.19	0	0	0	0	100
slnge	13.28	7.86	7.86	2.03	42.55	15.58	10.84	0	0	0	0	100

The probability matrix is viewed left to right. The first column shows the initial behaviors and the other columns along a given row show the percent probability of the first subsequent behavior.