SleepStats

Signal Solutions, LLC

User Manual

for SleepStats Version 4.x Base, Pro, and Lite

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Sleep Stats Data Explorer

Version 4

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Contents

Introduction

SleepStats software provides multiple ways to view and characterize rodent behavior data collected with **PiezoSleep**. The software includes functions that process multi-channel files created by the high-throughput data recording system **PiezoSleep** for efficient visualization and overlays of supporting data. The main functional features of this software are:

- Plotting graphical descriptions of sleep and wake behavior.
- Generating CSV files with statistics characterizing sleep and wake behaviors.
- Presenting classifier data with sensor signals to check signal quality.

There are 3 versions of **SleepStats 4: Pro, Base, and Lite**. The **Pro** version includes all the functionality described in this manual for mouse recordings. For rat recordings, however, a 3-state (Wake, NREM, and REM) classification feature does not yet exist in our commercial systems. The **Base** version includes all functionality for the 2-state sleep-wake classifier, as well as overlaying and exporting environmental data (if an environmental sensor was used during the recording). The **Lite** version has limited functionality and is useful for providing a simple summary of the sleep-wake and overlaying and exporting environmental data. It can be downloaded for free through our website. A chart in Appendix C lists all the features and distinctions between versions.

The same programs open files from either the mouse or rat **PiezoSleep** system recordings. The sleep-wake classification algorithms are different for mice and rats, but the program applies the proper algorithm based on the file extensions (or header information) of the recorded file. Since **PiezoSleep** systems classify short time intervals in the vigilance states noninvasively based on body motions, it only predicts the likelihood that the brain is in a particular state (sleep or wake) based on signal features generated by various postures and motions with our sensor technology. So ultimately this system assesses sleep-like or wake-like behavior. Performance of the classification algorithms is based on correlation with actual brain state based on invasive EEG/EMG signal scoring. Several validation experiments have been performed both internally and externally. For mouse systems correlations with EEG/EMG scoring are typically in the lower 90% range, while for rats the assessments fall in the mid 80% range. Appendix B describes the various validation experiments and performance results in more detail.

Installation

Download the **SleepStats** installer (usually in a zip file) from the URL provided by Signal Solutions and save it to a directory of your choice. Unzip and execute the downloaded file (or copy from a USB thumb drive if mailed out with the shipment). The installation script should identify *Signal Solutions, LLC* as the publisher in a query box, requesting permission to continue. If this is your first program installation, it will download library files from the MathWorks website (so you must be connected to the internet for the first installation). The transfer requires about a 1 GB download. In the rare event that the MathWorks server is slow, or the

internet traffic is congested, the download may timeout and give an error. In that case, wait a few hours and try again. Once fully downloaded, the installation script will continue with a prompt to suggest a folder to save the program files. You can change this if necessary. If you have no reason to set up a program in a different directory, just accept the default location (this is where Windows typically installs all its programs). For easier access to the program, select the **Add shortcut to Desktop** box. If you don't select this, the program will be available through your desktop start menu under **SleepStats..**. The installation script will then indicate that the program requires MATLAB runtime. Select the install option and the program will download the necessary files from the MathWorks server. This may take several minutes. Once installed, you can access the desktop icon or find the program **SleepStats** in the program start menu.

The first time the actual program is launched, it will contact our server to validate the installation on the computer that it is being installed on. A code should have been provided with the software purchase that will need to be entered when prompted. If you do not have the code, please contact Signal Solutions to have it sent to you. If you need to move the software to another system, contact Signal Solutions so the software can be released from the current machine to be revalidated on the new machine.

If you purchased or upgraded to the PRO version, you should have also received a link to download the 3-state file maker program. Once downloaded and unzipped, run the installation script. This will result in a program called **REM_NREM_Wake** (and a desktop icon if this was selected during installation). This program starts with a prompt to open the pressure signal files (binfb extension) and will generate a 3-state detection file indicating the vigilance state of the animal every 2 seconds. This file will end with the string **_3s.wss**. This program takes a while to run. On a typical PC, it requires about 10 seconds per hour per channel. So, an 8-channel recording for 5 days would take about 2.7 hours to run. Multiple files can be selected with the file select window. When the file select window is canceled, the program will start processing the selected files. Unfortunately, at this time the features used in this algorithm cannot be computed in PiezoSleep in real time. So, for 3-state analysis, this program must be run after PiezoSleep has finished the recording.

SleepStats Functions

This program can be used for checking the integrity of data, observing sleep-wake parameters in graphs, generating statistics, viewing the piezo signal, and creating CSV files for further analysis in a spreadsheet and database programs. Double-click the desktop icon or run it from the Windows Start Menu. On the first launch, **SleepStats** may take a few minutes to launch, this is typical.

File Menu

The File Menu, located in the top right corner of the app, shown below in Fig. 1, is used to open files and close the application.

Fig 1: SleepStats 4.0 main user interface window.

Clicking on **Open** (keyboard shortcut *Ctrl+O*) opens a file navigation window to select a feature vector file generated by **PiezoSleep**. By default, files listed in the select window are filtered so that only **FeatVecfb** (mouse) or **FeatVec** (rat) files appear. This can be changed in the file navigator window so feature vector files with extensions using different capitalizations, or non-fb feature vector files can be opened (this should not happen if using files directly exported from a current version of **PiezoSleep**). The **FeatVec** file contains the signal feature vectors generated by **PiezoSleep**, which are used to compute decision statistics and thresholds that determine sleep or wake states every 2-seconds over the entire recording. It may take a few minutes to open the file, depending on file size and computer speed/memory.

Close the program by clicking **Exit** (keyboard shortcut *Ctrl+X*) or closing the app in any other typical manner. A file with a **MAT** extension is created the first time a feature vector file (**FeatVec**) file is opened. By default, it contains settings for the graphic displays, environmental sensor-to-cage assignments (if using multiple environmental sensors), and the sleep-wake thresholds computed from the automatic threshold setting algorithm. These can be changed during a session. When closing the file or switching to a new one, an option is presented to save the changes made to the default parameters (in the **MAT** file). The next time this file is opened, it will load the settings that were saved in the previous session rather than the defaults. If this *MAT* file is deleted, the program will generate a new *MAT* file with the automatic threshold settings and original default values.

Left Panel - Changing Parameters

Once a file is opened, the "Select Channel" dropdown menu will be populated with the channel labels associated with the feature vector file, and the last save or default setting will appear in other text boxes, shown in Fig. 2.

Select Channel

Some of the plot and export functions are only available after a channel is selected from the dropdown menu. Once a channel is selected, relevant processing options and plots will be updated for that channel using the setting in the left panel. An example of the left panel is shown in Fig. 2a, where no channel has been selected yet. The text boxes and settings are explained in the following sections.

Sleep/Wake Decision Threshold

The current threshold value is applied to the decision statistics for distinguishing sleep and wake states. This threshold is calculated when the feature vector file is initially opened. A new value can be entered into this field, which replaces the previous value and updates all the relevant plots and statistics in the current session. The thresholds are set automatically, based on maximizing the decision statistic separation between the sleep and wake clusters. In most cases, this will be the best value. However, if data were collected in a noisy environment (i.e., from nearby machines creating mechanical vibrations), this may create odd patterns in the signal and the auto threshold algorithm may fail to work properly. Details on making threshold adjustments when odd patterns do exist in the data can be found in ***Appendix A***.

Minimum Bout Length Sleep/Wake

The minimum bout length is used to compute bout lengths based on consecutive minimum bout length intervals containing 50% or greater of the target behavior detections (detections are made every 2 seconds). The minimum bout length window is applied to recorded data in 2-second increments, and the first window encountered with 50% or greater sleep detections is marked as sleep. This starts the bout length count. As the window is incremented through the time series in 2 seconds steps, the bout length is increased while the new window contains more than 50% sleep. When an interval occurs containing less than 50% sleep, the bout length is terminated. 2 methods are applied for counting bout lengths; these are discussed in the bout length section. The smallest selectable minimum bout length is 2 seconds, which is the resolution of the decision algorithm. The default minimum bout length is 30 seconds, but this can be

(a)

(b)

Fig 2: Left panel options. (a) Default appearance, (b) onset options if Use Light/Dark Sensor Times selected.

changed in the settings panel. Typically, longer minimum bout lengths will result in reducing the number of shorter bouts and increasing the number of longer bouts in the count.

Bout Percentage Q Threshold

This option is useful for characterizing low-probability events. The threshold (in seconds) is applied to the sleep or wake bout lengths to compute the percentage of bout lengths greater than or equal to this value. The default is 256 seconds. This only affects the **Segmented Bout Stats Export** option for the **Qth percentile** output. This is particularly useful for characterizing extreme events. If long bout lengths are of interest, this statistic directly characterizes events in the *tail of the probability density curve*, rather than the median or mean, which describe typical values/behaviors.

Light & Dark Period Length in Hours

This option changes the number of hours for a *daily* cycle. This can be useful for running experiments where the light and dark times are not on a 24-hour cycle. This option is only useful if the recording was performed with a light-dark cycle that was **NOT** 24 hours. If *day* lengths are not periodic over the recording, you must export sleep/wake percentages to a time series and manually compute statistics of interest over the segmented periods. If an environment sensor was used in the recording, the **Pro** version will allow you to segment light and dark cycles based on changes in the light intensity automatically.

Minimum Bout Length Wake-REM-nREM (relevant for Pro Version only)

The minimum bout length for wake-REM-NREM is applied like the one for Sleep/Wake 2-state case but is applied to the 3-state analysis. So, for a bout length to be increment in time, the target behavior state must occupy 50% or more of the new minimum bout length interval. This minimum bout length setting for the 3-state is independent of the 2-state computations because for states like REM, a 30-second minimum bout length would typically miss most of the REM detections. Therefore, the default for the 3-state setting is 4 seconds, which will pick up many of the smaller REM bouts with is more typical of this state.

Light/Dark Onset Times

This is used to change the light and dark onset times of the recording. These parameters are set when the recording was started in **PiezoSleep** (saved in the header of the data file). However, this setting can be changed in **SleepStats** through the fields at the bottom of the left panel shown in Fig 2. This may be useful if the original values were determined to be incorrect, or if the user wants to explore other ways to segment the data on a daily cycle. The onset times are in a 0-to-24-hour format. If a change is made to these parameters, then all the plots involving light and dark phase segmentation will update to the new times, and subsequent exports will use the new times as well.

If environmental sensor data is available, the **Use Light Sensor Times** button will be enabled, and if selected, the light and dark onset times will use detected transition the light data from the environmental sensor to determine light and dark onset times. Once this button is selected, the Light/Dark Onset times box will change to look like Fig 2b. Selecting **Change Light Thresholds** will allow the user to change the "Light/Dark Threshold (Lux)", default 50 Lux. When

the light level crosses this threshold, an onset time is assigned. A crossing in the positive direction assigns a light onset, and a crossing in the negative direction assigns a dark onset. To prevent rapid light flicker or noise/power fluctuations from erroneously setting an onset (sometimes flicker occurs when lights are turned on), a "Minimum Light/Dark Interval (min)" value is also applied, such that the current light state must be on for at least this amount of time before allowing a change to the next state. The default is 0.25 minutes (15 seconds).

Center Panel - Plots

The Center panel presents the plots generated by **SleepStats**. The plots are separated into 3 groups, which are denoted in the upper row of tabs on the user interface. The **Base** and **Lite** versions will only have 1 upper tab the *Sleep Stats Plots* (see Fig. 3). The **Pro** version will show all 3 groups as tabs in the upper row, as displayed in Fig. 3. These groups and their associated plots are summarized below. The subplot tabs appear in the second tab row on the user interface.

- Sleep Stat Plots: 2-state sleep-wake decision plots (**All** versions)
  - Percent Daily Sleep (**All** versions)
  - Percent Daily Sleep in Light & Dark (**All** versions)
  - Sleep-Wake Decision Statistic (**All** versions include environmental sensor overlay, but pressure sensor data and breath rate overlays are only available in **Pro** and Base, and activity level overlay is only available in **Pro**)
  - Sleep-Wake Decision Histogram (**All** versions)
  - Percent Sleep/Wake Over Sliding Interval (**All** versions)
  - Sleep-Wake Bout Length Histogram (**Base** and **Pro** versions)
- 3-State Stat Plots: Wake, REM, and NREM decision plots (**Pro** version)
  - Percent Wake-REM-NREM
  - Percent Wake-REM-NREM in Light & Dark
  - 3-State Decision Statistic
  - Wake-REM-NREM Bout Length Histogram
- Activity Stat Plots: Circadian Activity patterns (**Pro** version)
  - Actogram Plot

Many of the plots have specific parameters affecting their appearance calculations can be changed. These parameters can be edited within the tab associated with the plot.

Each plot image can be saved in one of two file formats: PNG or EPS (vector format). This can be done by right-clicking on the plot area and selecting ***Save Figure as the desired output format***. The plots are saved to the same folder as the feature vector file.

SleepStat Plots

If *Sleep Stats Plots* are selected in the top tab row, a series of graphical representations of the data can be observed in the lower tab row. These plots are based on the 2-state sleep-wake decisions.

Percent Daily Sleep

The Percent Daily Sleep plot, shown in Fig. 3, computes the percentage of time spent sleeping over an integer multiple of days, based on the **Light & Dark Period** setting (default is 24 hours). In a multi-day recording, fractions of a day are not used in the computation. Full days are counted from the first light onset to the last light onset. The time before the first light onset and after the last light onset are excluded from the computation in the graphic. Partial days in the sleep percentage computation can bias the result for *daily sleep* depending on the light-dark state of the partial days included. However, when the recording time does not include two light onsets (a short recording), in which case the whole file is used in computing the sleep percentage. If the partial days of the experiment are important, then the **Percent Sleep/Wake Over Sliding Interval** plot tab can be useful for a quick examination of sleep or wake behavior near the beginning and end of the recording. A more precise way is to export the percent sleep over shorter intervals using the **Percent Sleep per User Defined Interval** option, as it can generate sleep percentage over user-defined intervals from any beginning to any ending time within the recording period (see the export option section for more detail).

Fig 3: Percent Daily Sleep Plot with 4 channels.

Percent Sleep in Light & Dark

The Percent Sleep in Light & Dark plot segments out all the light and dark intervals from the first light onset to the last and presents the average over all days in side-by-side bar plots. This is illustrated in Figure 4.
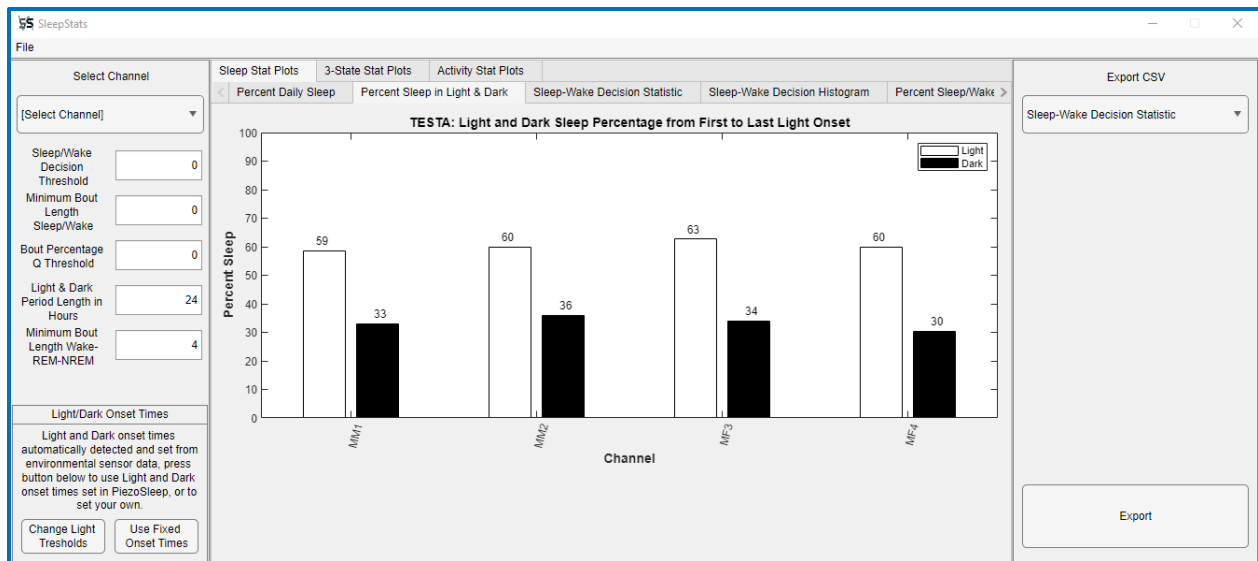
Fig 4: Percent Sleep in Light & Dark Phases

Sleep-Wake Decision Statistic

The *Sleep-Wake Decision Statistic* plots the time series of the decision statistics, which are measures of sleep-state likelihoods, derived from the signal features of the pressure signal every 2 seconds. The more positive the number, the more likely the animal was sleeping. This is useful to determine if the threshold needs to be adjusted or if something unusual happened in time (deceased animal, bad sensor, noise, interference, etc.), in which case, the likelihood patterns would change. Figure 5 is an example of the decision statistic for the channel labeled MM1. The black vertical dashed lines correspond to dark onset times and the cyan vertical dashed lines correspond to light onset times. The time axis is in hours, relative to the first day the experiment started. So, hour 0 corresponds to 12 a.m. on the day the experiment started, and hours are increased linearly throughout the entire recording. The green horizontal line is the Decision Threshold, where a decision statistic greater than this value will result in the interval being labeled as sleep, otherwise wake. The Decision Threshold can be changed by editing the first textbox in the left panel, labeled *Sleep/Wake Decision Threshold*. The initial threshold value is from an automatic threshold algorithm that adjusts the threshold, based on the clustering of these statistics in a 2-state mode. These threshold values are stored in the *MAT* file in the same directory as the recording. If changes were made during a session, they can be saved when exiting the program (or switching to open a new file). The next time this file is opened it will apply the saved values. If not saved, the thresholds will be reset to the values from the automatic threshold setting. Once a channel is selected and plotted, the overlay options below will be available for plotting along with the decision statistics.
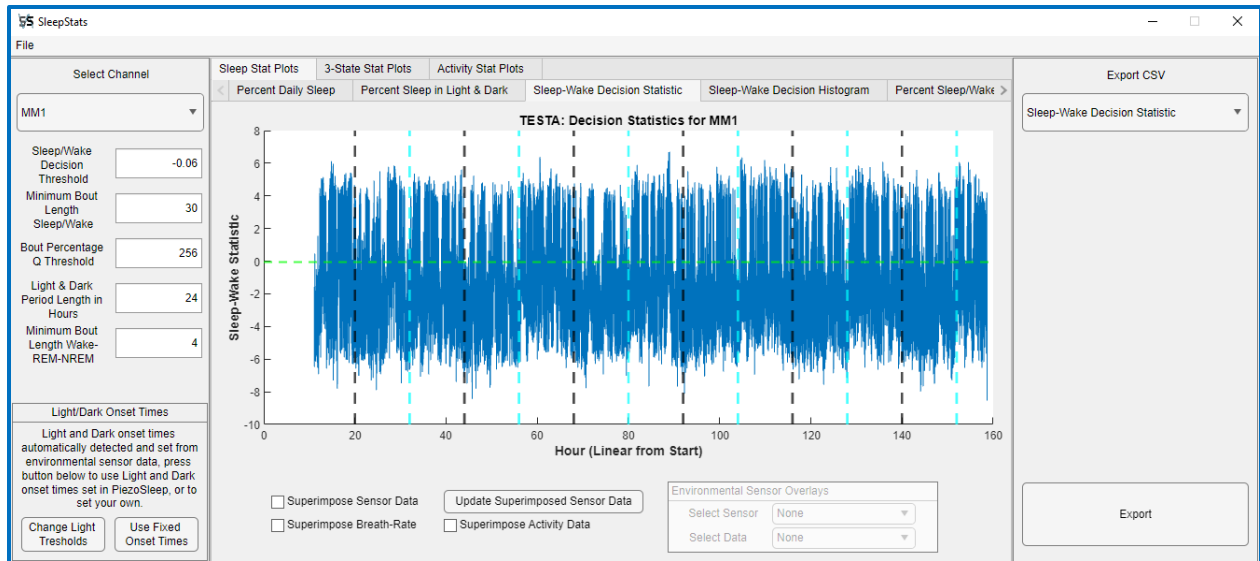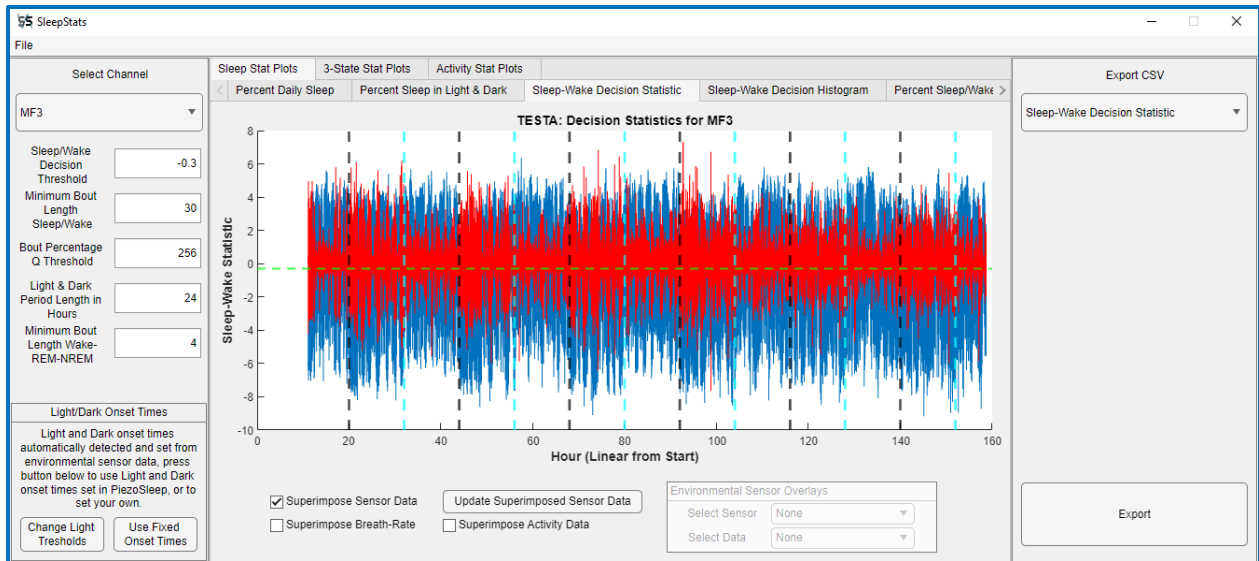
Fig 5: Sleep-Wake Decision Statistic
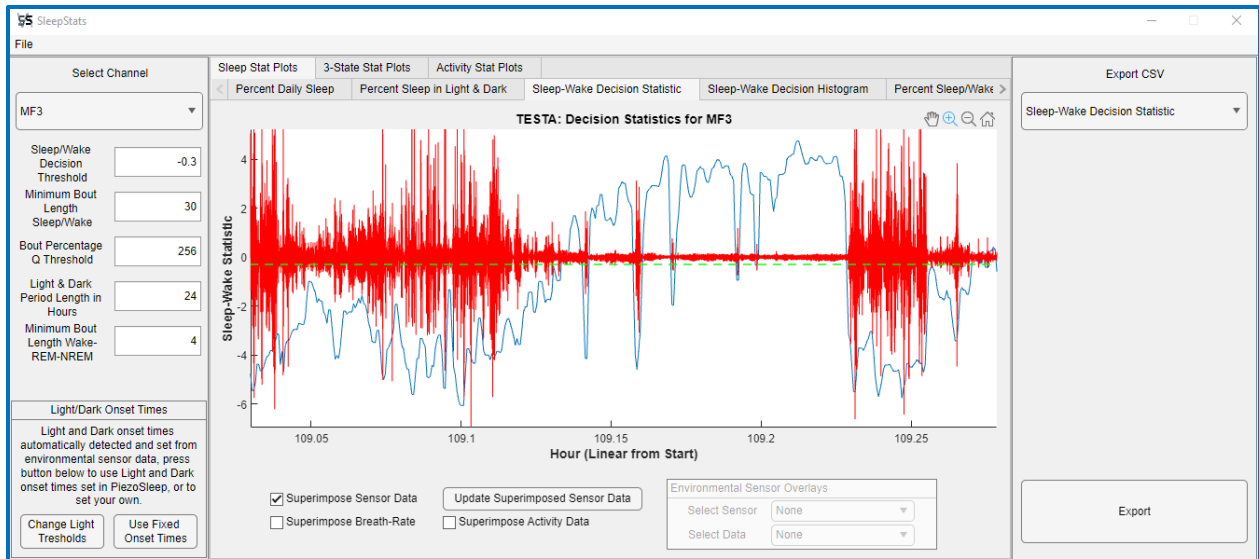
Superimpose Sensor Data

This plots the raw sensor data, which are pressure values resulting from the animal interaction with the cage floor and are contained in the **bin(fb)** file. Since this file contains 120 samples every second, the signal is subsampled depending on the range of the plot time scale. When zooming into smaller time scales, the resolution needs to be increased to see a proper representation of the signal. This is done by clicking the *Update Superimposed Sensor Data* button. It is important to do this, as the lower resolution can look very different at smaller time scales since it misses important details. It is usually more efficient to zoom into an area of interest first (based on the decision statistics) and then select the overlay option. Figures 6a through 6c illustrate starting with the full-time range scale (low resolution) and zooming in and updating to see finer resolutions. Figure 6a shows the result of selecting the *Superimposed Sensor Data* when the full 160-hour axis is displayed. Figure 6b shows the result of zooming into an interval of about 0.2 hours (or 12 minutes). The waveform in this case is not accurate because it is still at the lower resolution level. Figure 6c shows the result after clicking the *Update Superimposed Sensor Data* button, where now more detail can be seen commensurate with the plotted time interval.

(a)

(b)

(c)

Fig 6: Sleep-Wake Decision Statistic with superimposed sensor data: (a) Initial overlay for whole recording at low resolution, (b) Zoomed-in Sleep-Wake Decision Statistic with superimposed sensor data (not updated, still at low resolution), (c) updated superimposed sensor data at a commensurate resolution to the time scale.

The pattern in Fig. 6c illustrates how the signal features impact the sleep detection algorithm. Note the intervals with large amplitude swings of the pressure signal correspond to negative decision statistics. This is characteristic of wake, where strong and rapid body motions result in dense and high amplitude signals. When the amplitudes decrease and become more regular, the decision statistics become more positive and cross the threshold to result in sleep detections. The longer sleep bouts are observed in the contiguous stretch of low-level signals with regular amplitudes (near constant signal envelope). This represents a stillness consistent with sleep behavior. If zoomed in even more on the low-level amplitudes (not shown here), a regular pattern is observed, corresponding to the breathing detected from the thorax motion on the cage floor. This regular pattern has a high correlation to the sleep state. Note near the end of this plot (after hour 109.3) there are low-level signals, but the amplitudes have more irregularity than the sleep-detected areas. This is more consistent with conscious behavior in a low-activity or rest state, but not sleep.
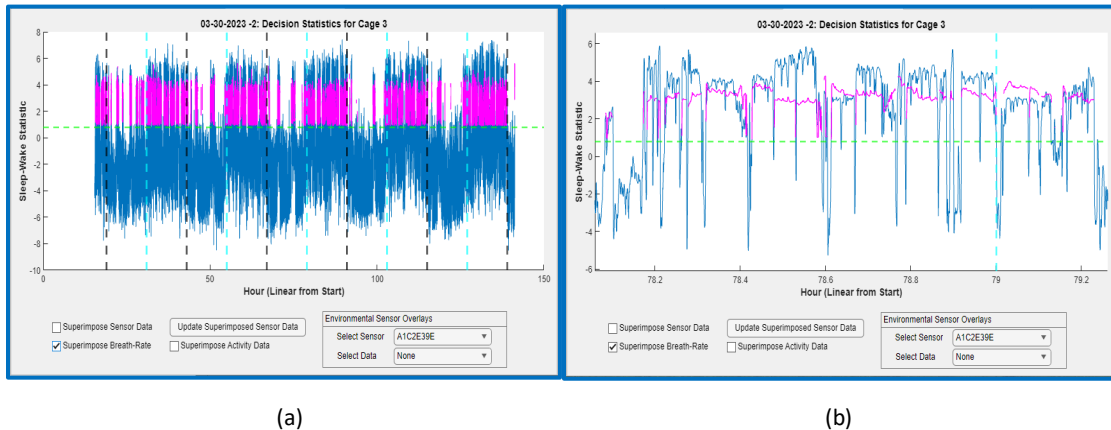
Superimpose Breath Rate

Fig 7: Sleep-Wake Decision Statistic with superimposed breath rate: (a) Full-time scale, (b) zoomed-in to see rate changes.

The accurate estimation of breath rates requires a good signal-to-noise ratio, such the breathing signal is not corrupted by the noise signals (vibrations from other sources outside the cage). While the Sleep-Wake classification continues to operate accurately for higher noise levels because of the redundancy of signal features used, the accurate estimate of a respiration rate will degrade for lower levels of noise that are required for sleep and wake detections. Therefore, film sensor cages are recommended for more accurate breath rate estimations. The 2-sensor adapt-a-base system typically does not have a high enough signal-to-noise ratio for consistently accurate breathing estimation.

The ***Superimposed Breath Rate*** option overlays the breath rate estimates during detected sleep periods. These estimates are stored in the file ending with the string ***_br.Feat***. This option is unavailable if this file is missing.

Superimpose Activity Data (only available for Pro version)

This option overlays the activity level estimates stored in the file ending with the string ***_at.Feat***. This option is unavailable if this file is missing. Activity levels are set to 0 during periods of detected sleep and a level is estimated during periods of wake with a calibration process described in the Activity Stats Plots section The more rapid and dense the movements that create pressure on the cage floor, the higher the activity level. An example of the activity level overlay is shown in Fig. 8 for a 40-minute segment. The additional overlay of the pressure signal (Fig. 8b) demonstrates a relationship between the pressure signal and activity level.
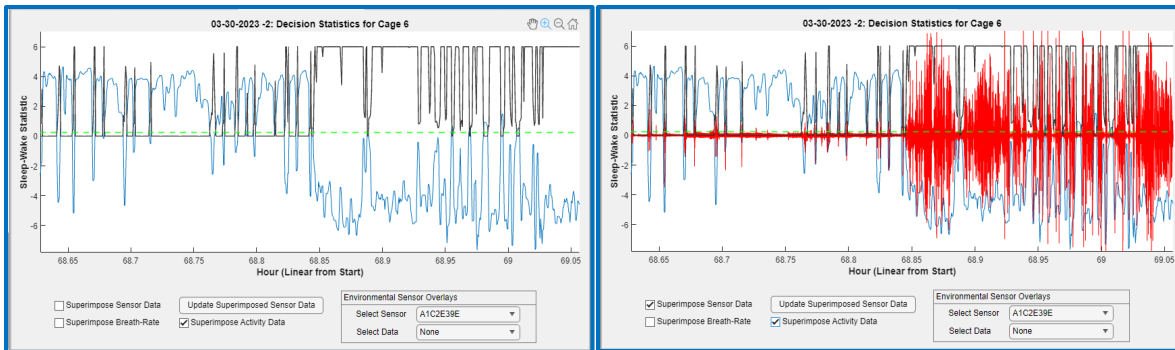
(a)   (b)

Fig 8: Sleep-Wake Decision Statistic with superimposed activity data: (a) without pressure signal overlay (b) with pressure signal overlay.

Environmental Sensor Overlays

If an environmental sensor was used during the recording, then the following parameters are recorded from the cage environment every 2 seconds:

- Light Level (Lux)
- Temperature (Degrees Fahrenheit)
- Relative Humidity (Percent)
- Pressure (Inches of Mercury)

The panel shown in Fig. 9 demonstrates overlays of these parameters on the decision statistics. If an environmental sensor from Signal Solutions was used during the recording, a file with the extension ***Env*** will be present in the folder with the other data file created. If this file is not present, this panel will be unavailable. The upper ***Select Sensor*** drop-down menu allows the user to select from multiple environmental sensors, if available, the lower ***Select Data*** menu selects which parameter will be plotted. The environmental sensor data is plotted using the right y-axis, so the different units affect the plot scale independently. This way the sensor data and decision statistics can be better observed in the same window. If the zoom option is on, clicking on either the right or left axis will determine which plot is affected by the zoom actions.

If multiple environmental sensors are used, a menu will appear with options to associate different sensors with the cages they are to be associated with.
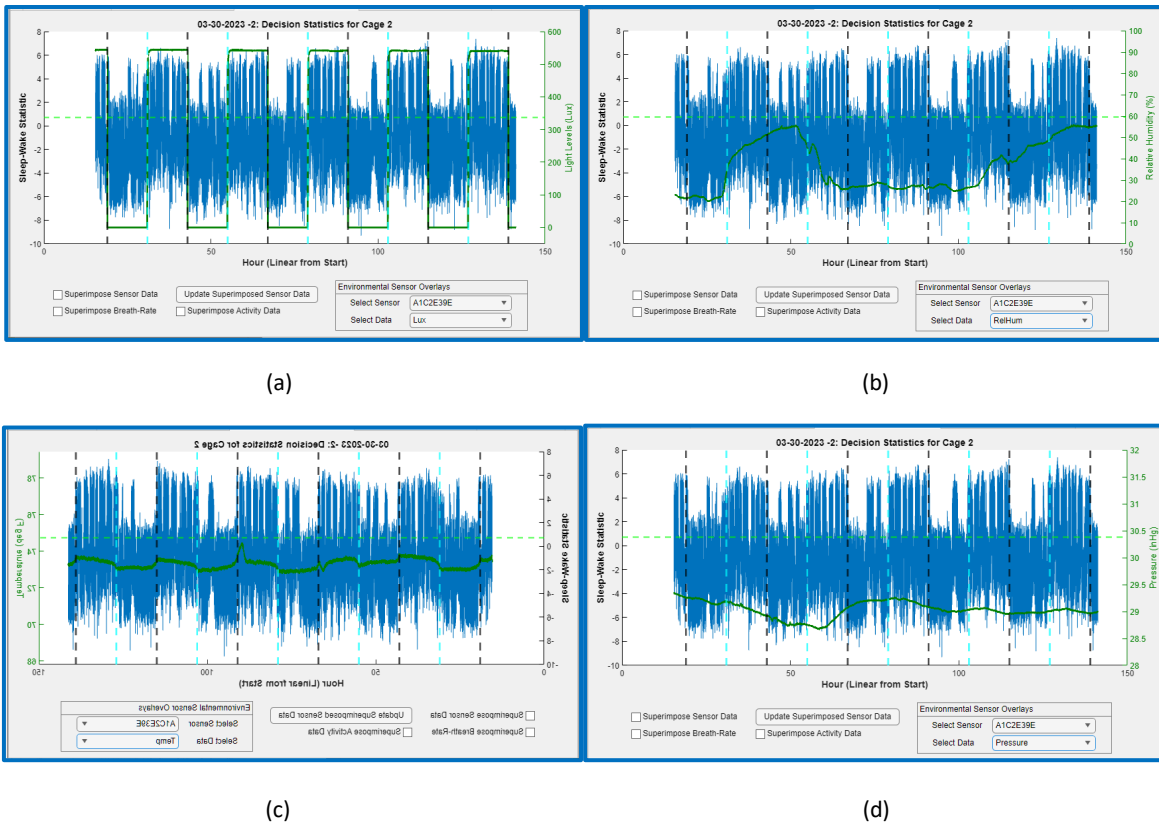
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Fig 9: Sleep-Wake Decision Statistic with Environmental Sensor Overlays. Y-axis on the right is in units of the environmental parameter plot in green: (a) Light Levels, (b) Relative Humidity, (c) Temperature, and (d) Pressure.
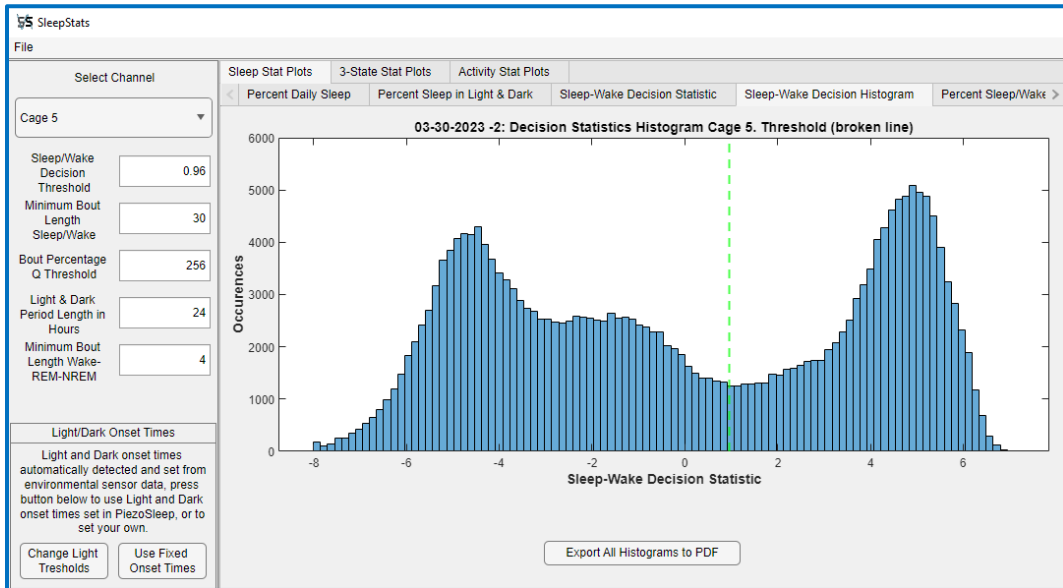
Sleep-Wake Decision Histogram

The Sleep-Wake Decision Histogram is useful for assessing how well the classifier distinguishes between distinct sleep and wake behaviors. For an animal with typical sleep and wake behavior patterns, decision statistics over the course of 24 hours should cluster toward negative values for wake and positive values for sleep. A bimodal histogram of the decision statistics usually indicates that the algorithm worked as expected and allows for a relatively accurate auto threshold setting to separate sleep and wake behavioral intervals. Figure 10 shows examples of histograms with expected bimodal peaks for decision statistics. The adaptive threshold is also shown located near the saddle point (green vertical line). For a typical recording greater than 24 hours, clustering to either side of zero suggests reliable results. However, for more varied behaviors that can occur during the wake, the histogram may not show a sharp peak or can even be a very broad spread of values, depending on the variety of activity during wake. In comparing Fig. 10a to 10b, sharper, more bimodal peaks are observed in Fig 10a, while the case of 10b shows almost 2 peaks for the wake behavior alone. Patterns like this can indicate multiple distinct wake behaviors, as in the case of an animal becoming more lethargic for some days or even persistent room/platform vibrations. This can sometimes cause the automatic threshold to be set to less than optimal. In this case, it would be a good idea to check the ***Sleep-Wake Decision Statistic*** time series plots (previous tab) to make sure the sleep-like and wake-

like waveforms are being separated properly. See *Appendix A* for information on adjusting the sleep-wake decision threshold.
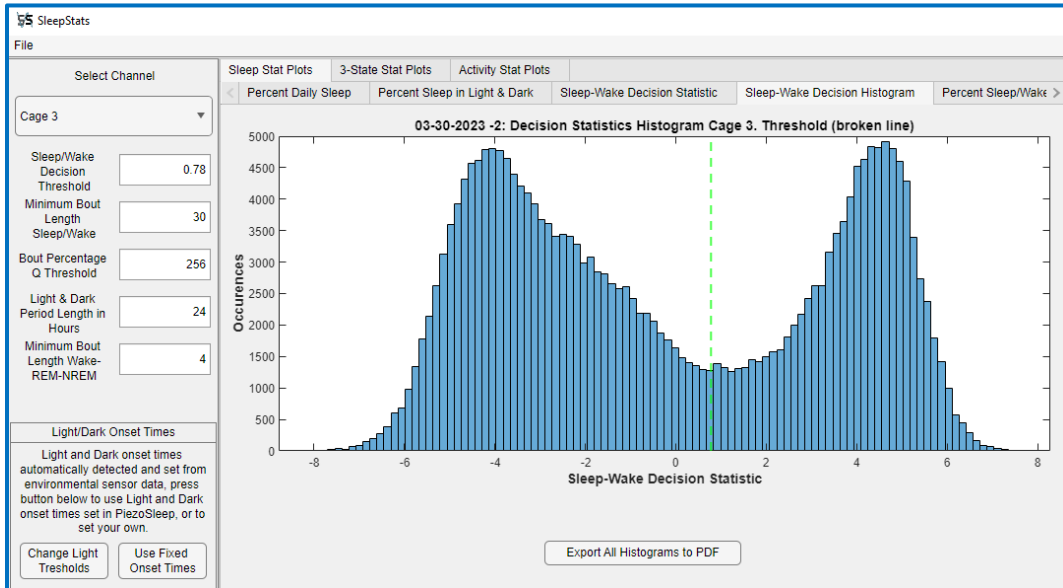
(a)

(b)

*Fig 10: Examples of bimodal decision statistic distributions, suggesting a good separation between sleep and wake activity.*

Examples of histograms that are not consistent with recordings of typical sleep and wake behaviors are shown in Fig. 11. These were empty-cage recordings, and they show how the decision statistics respond to noise and ambient vibrations in the environment. Figure 11a

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shows a typical response to noise that remains relatively consistent over the whole recording. Note the unimodal Gaussian-like form showing no distinct behavioral changes. Figure 11b also shows the response to noise, but the noise pattern changes over the recording period, likely from the ventilation system or ambient vibrations from a machine that turns on and off over various time intervals.

For more convenient inspections of all the histograms, the **Export All Histogram to PDF** button on the bottom can be used to create a pdf file with images of the histogram from all cages. This is less tedious than selecting each cage individually to view in the SleepStats 4 user interface. The pdf file will be given the same base name as the recording file with the text **sensor_histogramFigures** appended and will be in the same folder as the *FeatVec(fb)* file.
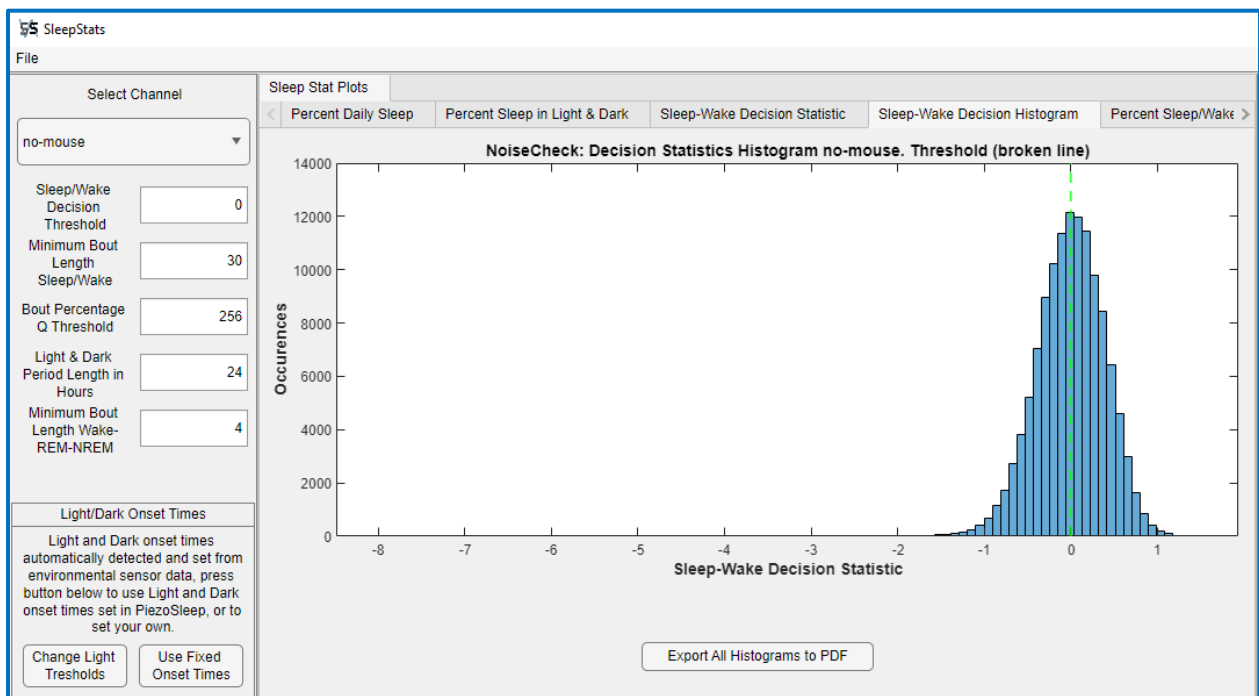
(a)
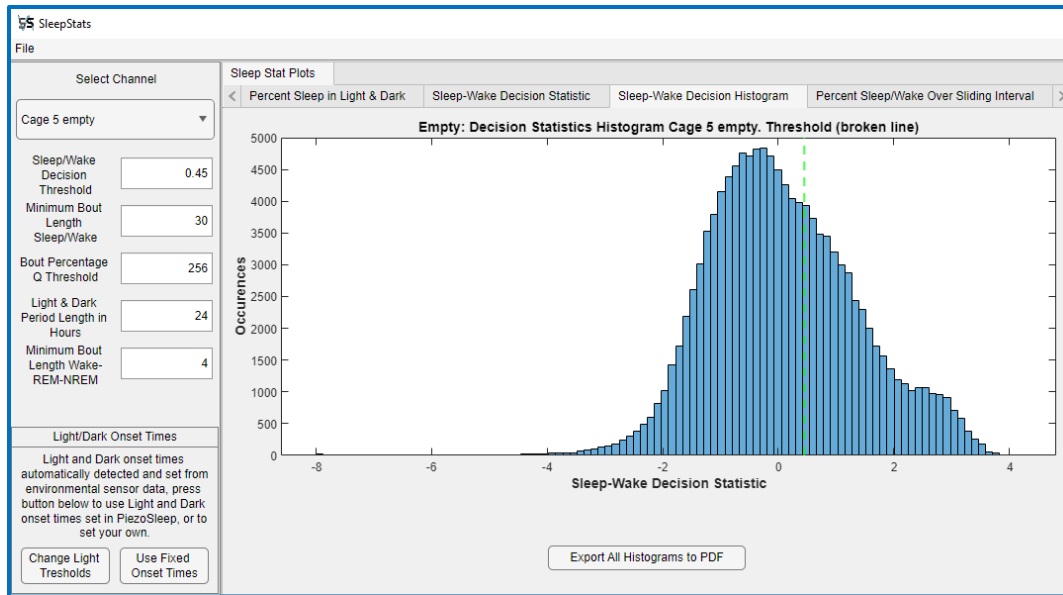
(b)

Fig 11: Noise-only histograms (top) from a well-isolated cage, and (bottom) from a cage with non-sleep loke interference patterns

Empty cage tests can be run from time to time to see if some environmental noises/vibrations can potentially interfere with the sleep-wake classification. If the histogram has an odd shape, the cage placement and electronic connections should be investigated for potential issues. Make sure cables are seated well into their ports, and make sure there are no vibrations from machines that are strongly coupled to the cage platforms. Also, other sources of noise include air currents from HVAC vents blowing directly on the cages, and cages on unsteady shelves or uneven surfaces below the cage that could cause the cage to rock.

Percent Sleep/Wake Over Sliding Interval

The *Percent Sleep/Wake Over Sliding Interval* option shows the percent sleep or wakes over a sequence of time intervals that are incrementally advanced (sliding window) over the entire data segment. This graphic is good for looking at the response to each of the light and dark onsets or some other intervention or event in the experiment. The larger interval removes smaller variations (as seen in Fig. 5) to bring out more persistent trends. Fig. 12 shows an example of sleep percentage over 2-hour overlapping intervals, computed every 0.2 hours (12-minute increments) over a 9-day recording. The time index denotes the midpoint of the sliding interval. The increment between intervals and the interval duration can be changed in the text boxes below, as well as switching between sleep or wake percentages.
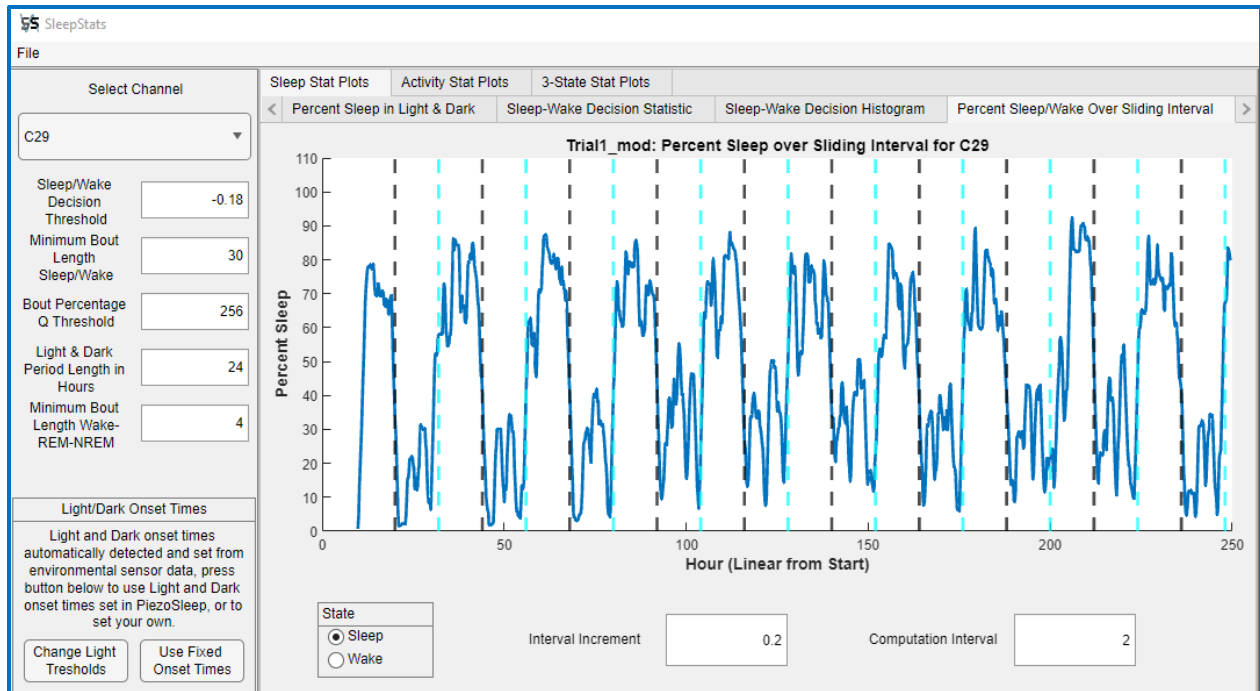
Fig 12: Percent Wake Over Sliding Interval plot

Sleep/Wake Bout Length Histograms

The *Sleep/Wake Bout Length Histogram* shows histograms of sleep and wake bout lengths. A bout length is an interval of time when the animal remains in the same state. There are several options available to control how bout lengths are computed and how the histogram bin values are presented. These options can be selected in the panels that appear with the graphic in the user interface panels shown in Fig. 13. The first option (left in the bottom sub-panel) determines how the histogram is computed relative to the light and dark phases of the recording. One option creates the bout length bins over all 24-hour segments from the first light onset to the last light onset, and the second option bins the bout lengths separately in the light and dark phases. If two light onsets are not included in the recording, then the whole recording is used. The bout lengths are binned over logarithmically scale ranges (bin range is double the previous range). The next box in the panel selects the target behavior. Sleep and wake bout lengths will not necessarily be complements of each other depending on the computation method used (described below) and the minimum bout length interval. An animal state is assigned to the times of minimum bout length interval if the interval contains 50% or more of the target behavior.
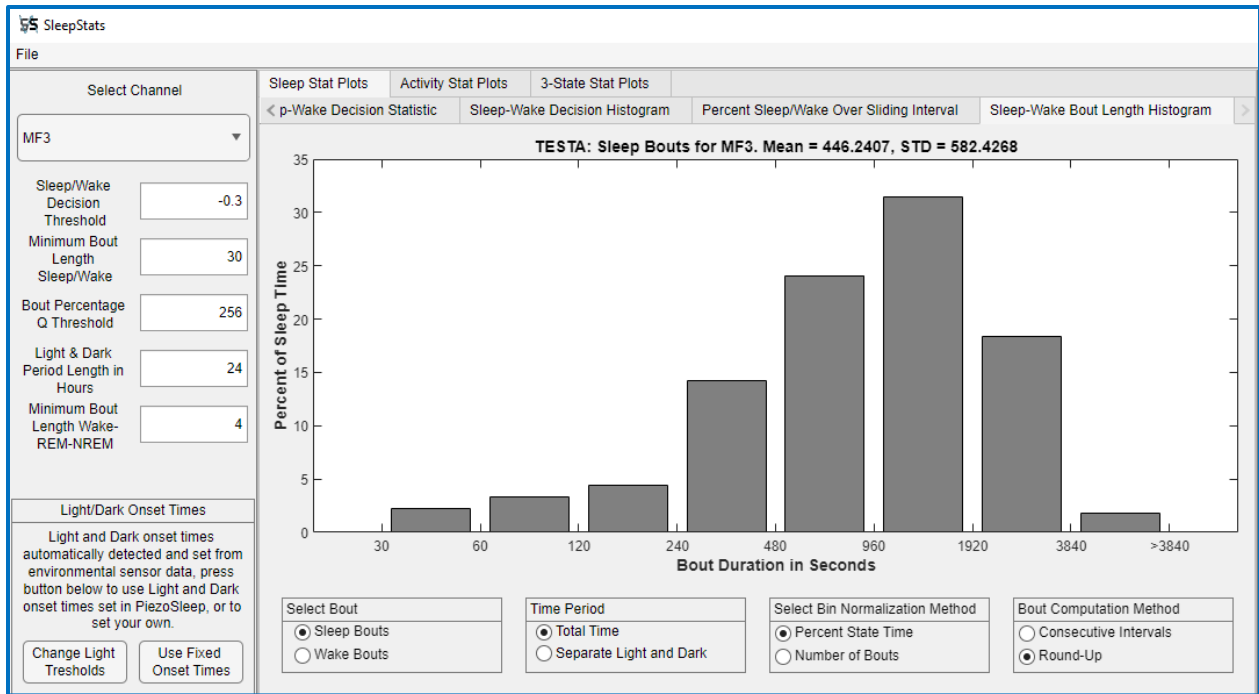
(a)
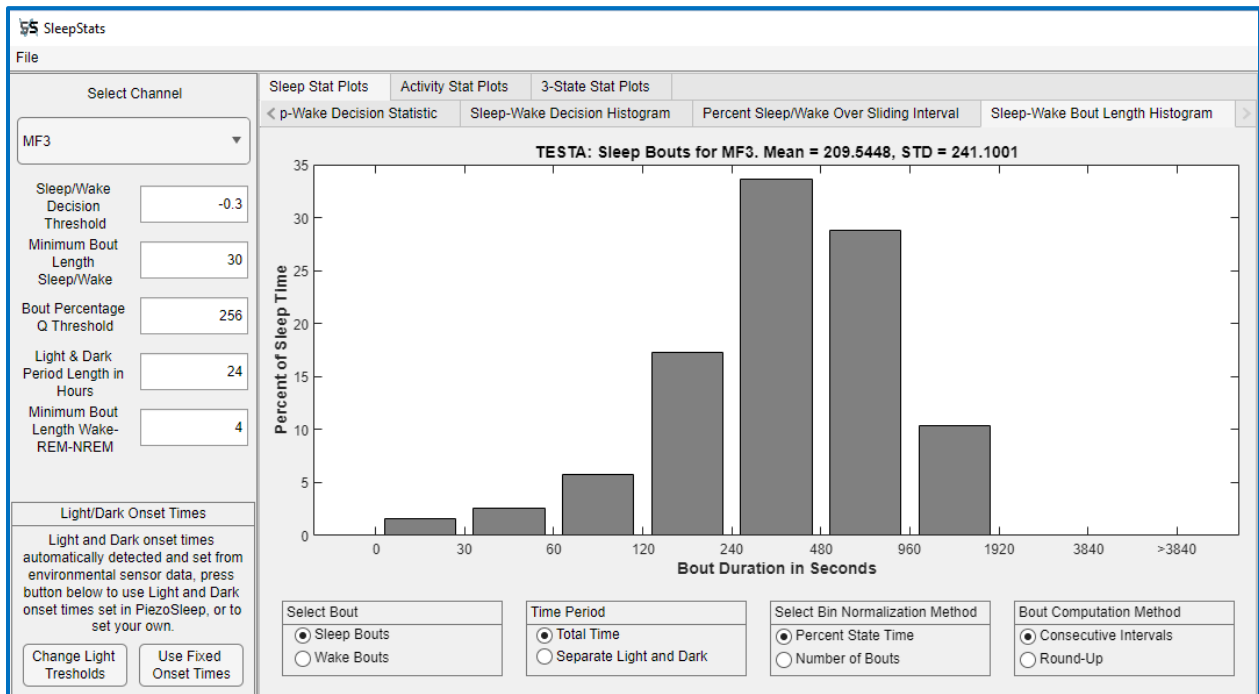
(b)

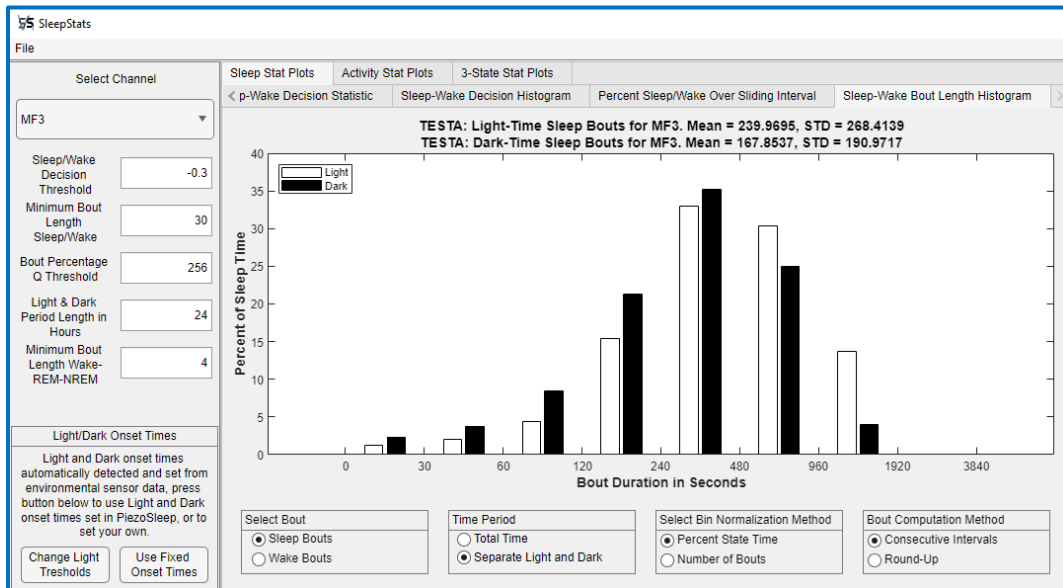Fig 13: Sleep Wake Bout Length Histogram, normalized over the sleep time independent of whether it occurred in the light or dark phase. The bout length computation methods applied are the: (a) round-up, and (b) consecutive intervals.

An important parameter in computing the bout lengths is the *Minimum Bout Length*, which can be set in the left panel. Note there are 2 minimum bout length text boxes: one for the sleep-wake (2-state decisions) and the other for the 3-state Wake-NREM-REM bouts. Bouts of a given behavior are computed by counting contiguous intervals of the detected behavior. While PiezoSleep provides a behavioral label every 2 seconds, it is not recommended to use the 2-second intervals to compute bout lengths. The occurrence of isolated instances of stirring or twitching during sleep bouts will typically cause wake detection during a sleep bout, where the animal is briefly aroused and then returns to sleep behavior. For many studies, the short occurrences of the non-target behavior are not significant since a definite transition into another behavioral state did not occur. For this reason, *SleepStats* has a *Minimum Bout Length* parameter that controls how the bout start and end are determined more robustly. In addition, a minimum bout length that includes a larger number of 2-sec detections improves robustness against isolated errors in the sleep-wake classification. However, it also reduces the resolution to shorter breaks in a vigilance state.

There are 2 ways SleepStats computes bout lengths. The method can be selected from the lower panel as either *round-up* or *consecutive intervals*. Figure 13 shows an example of computing bout length histogram by these 2 methods. For both methods, the minimum bout length interval is incremented every 2 seconds through the series of detected states while performing a test on the detection in that new interval. If the interval contains more than 50% of the target behavior, the interval is labeled with that behavior. For the **round-up** method, the whole interval associated with each time sample is labeled with the target behavior (thus, no bout can now be less than the minimum bout length interval). Then, the lengths of all consecutive labels are computed and binned for the histogram. So, in this case, bout endings are determined when there is a sustained transition to a different behavior. For the **consecutive intervals** method, consecutive labels for the interval centers are counted without rounding up to the full minimum bout length. So, for example, if an isolated minimum bout length interval passed the greater than 50% rule and the interval before it and after it did not, it would just be a bout length of 2 seconds. This method counts the number of consecutive minimum bout length intervals and multiplies by 2 seconds (the increment of the minimum bout length interval). The latter method results in shorter bout lengths as it is more sensitive to shorter intervals of the non-target behavior. These differences are observed in the histograms of Figure 13.

Bout length estimate robustness to errors also increases with increased minimum bout length; however, at the loss of detecting shorter actual bouts. Given an expected error rate of 5 to 10 percent on the sleep-wake decisions, using a window with the highest resolution (2 seconds) results in the bout length estimates that are strongly affected by that error. When the minimum bout length is set to 30 seconds (15 detections per interval), it is highly unlikely that eight errors will occur to falsely start or end the bout computation. The probability of a bout ending or continuing in error (using the higher error estimation of ten percent), is 3.5×10^{-5}, which comes to one error every 16 hours. Therefore, the default time is set to 30 seconds, but this can be changed using the **Minimum Bout Length Sleep/Wake** textbox on the left panel.

The normalization of the histogram bin values can be changed in the lower panel: *Select **Bin Normalization Method***. The default is to normalize over the selected state (*Percent State Time*). In this case, the bin values will add up to 100% and represent the percent of the sleep behavior time that was spent in that bout range. The second option, ***Number of Bouts***, shows the number of intervals detected in each bin range. Figure 14 shows an example of the 2 different normalization methods for sleep bouts computed over light and dark phases, using the consecutive interval method. Fig. 14a shows the normalized state time, and Fig. 14b is simply the number of bouts. Note how the histogram shapes are dramatically different. For example, Fig. 14a shows the lowest amplitudes in the 0 to 4-second range, indicating that the animal spent only about 2 to 3 percent of sleep time in these short bouts, while Fig. 14b shows almost the highest amplitudes in this same range. This is to be expected since the state time normalization converts the number of bouts to time (adds up bout lengths). So, these lower bout intervals length, though many add up to less time.

(a)
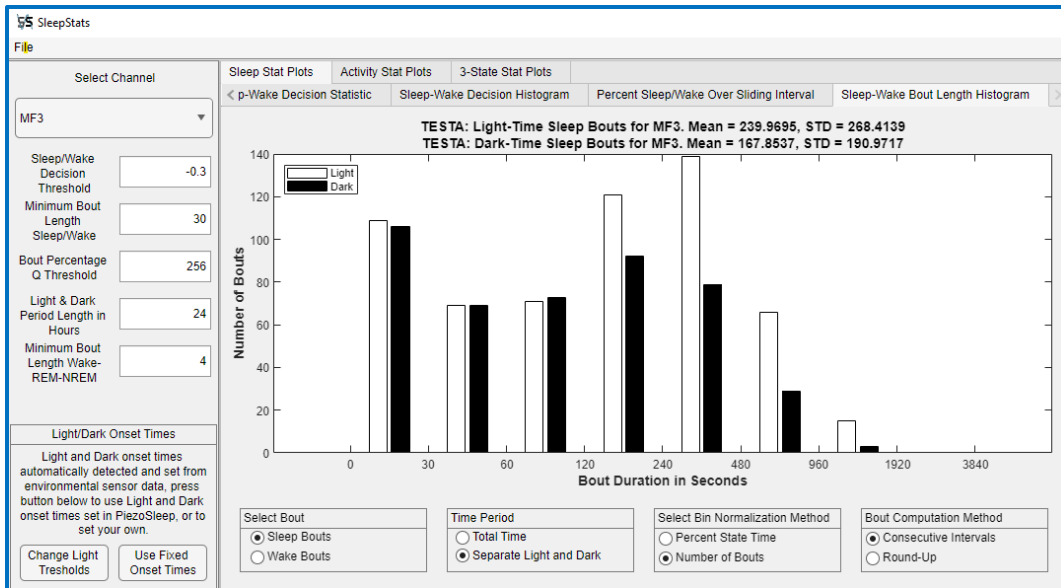
(b)

Fig 14: Sleep Bout Length Histograms examples segmented into light and dark phases: (a) normalized over the sleep state time, (b) number of bouts.

3-State Stat Plots – **PRO Version Only**

If the 3-state file was generated (the file ending with string _3s.wss), an upper row tab labeled *3-State Stat Plots* will be visible. The plots under this tab present similar options for the 2-state sleep-wake analysis. These plots are based on wake, REM, and NREM detections stored in the *_3s.wss* file, which is generated by the 3-state file maker program called *REM_NREM_Wake*. If you have SleepStats 4 Pro and do not have this program, please contact Signal Solutions for the installation script.

There are some differences in the settings panel on the left of the user interface. The 3-state analysis still applies the original 2-state sleep-wake detection algorithm, and then the detected sleep intervals are separated into REM and NREM based on the detected states in the *WSS* file. So, changes in the Sleep-Wake Decision Threshold will change the number of sleep intervals detected, and that will impact the number of REM and NREM detections. The Minimum Bout Length Sleep/Wake setting is only applied to the 2-state plots and has no impact on the 3-state computations. The rest of the settings are applied to the 3-state statistic computations as described in the 2-state Sleep Stats Plot sections.

Percent Wake-REM-NREM

Analogous to the percent daily sleep or wake (which are complements), this graph shows the percentage of time the animal spent in each of the 3 vigilance states, selected from the lower panel shown in Fig. 15.
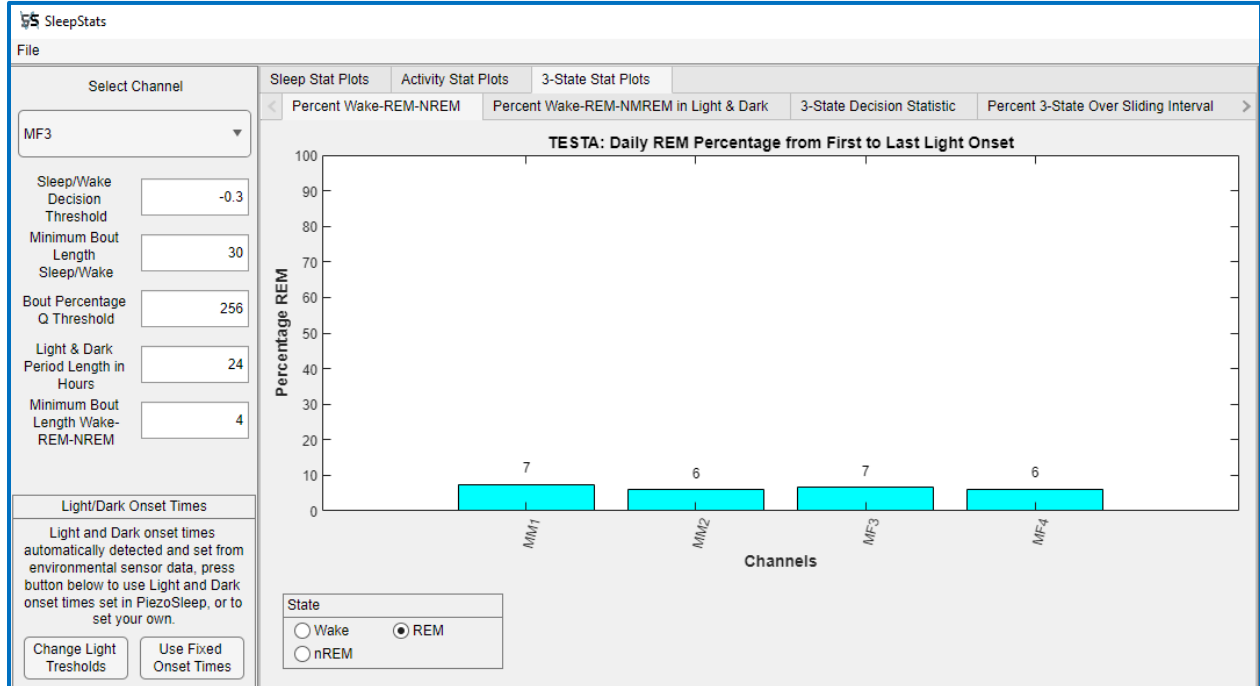
(a)
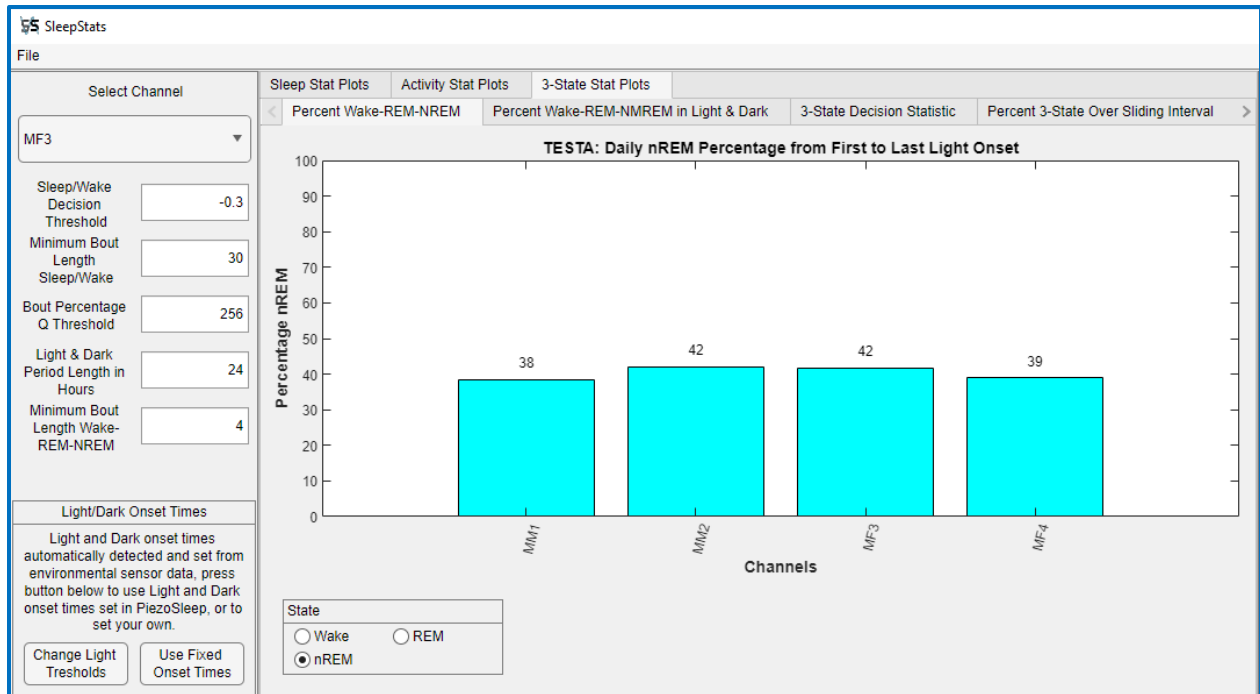
(b)

Fig 15: 3-state percent daily sleep (a) Percent REM (b) Percent NREM.

Percent Wake-REM-NREM in Light & Dark

This tab is analogous to the 2-state panel. Figure 16 shows an example of a recording that only includes one full day (24 hours) after the first light onset. If a full day from the first light onset is not in the recorded file, this graph cannot be generated since one full light phase and dark phase are not available for comparison.
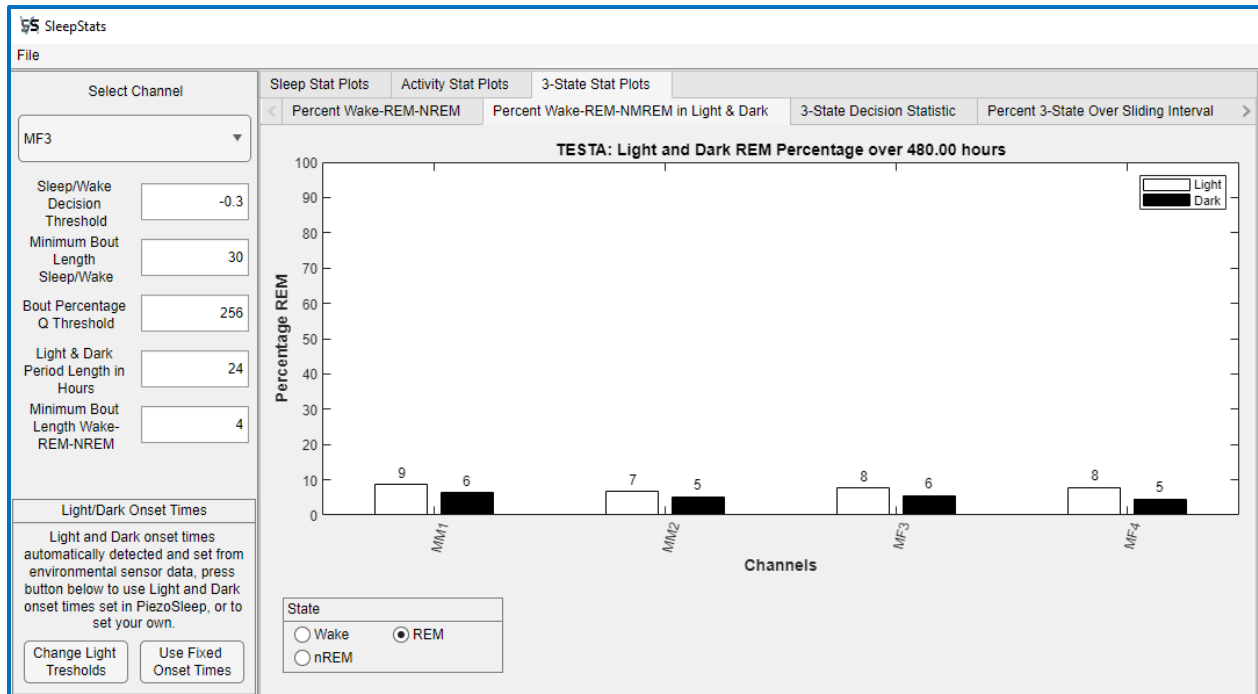
Fig 16: Daily Percent REM in light and dark phases

3-State Decision Statistic

The decisions for the 3-state algorithm are made on all sleep detections from the 2-state algorithm, using a decision tree on signal features primarily related to regularity and amplitudes of the breathing signal. As a result, there are no simple decision statistics where a threshold can be applied to change the sensitivity/specificity between the states. Therefore, the 3 states are plotted out in 3 discrete levels as a time series (rather than a continuous decision statistic). Note the left y-axis in Fig. 17 has the 3 discrete vigilance states labeling the tick marks, and the level of the blue line denotes the decision. The pressure signal can be overlaid to view the pressure signal corresponding to its detected state. An example of this is shown in Fig. 17a. For long recordings, all that is observable at this resolution is activity bursts at dark onset over the daily cycles. To observe the actual signal dynamic associated with each detection, it is best to zoom in to where the transitions between the 3 states can be observed as in Fig 17b. At this resolution, the signal envelope can be observed, and the blue line, located at the REM level of the envelope, becomes a little more irregular than signals corresponding to the NREM level. And the wake level corresponds to larger and more irregular amplitudes.

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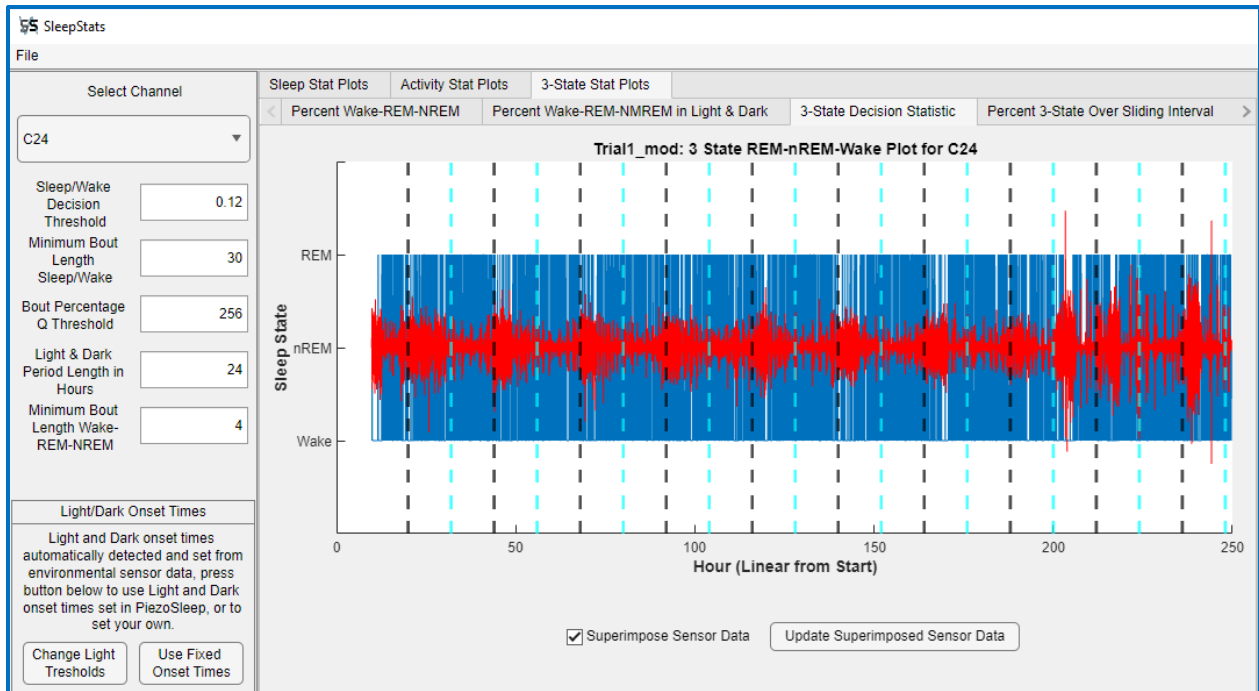
(a)
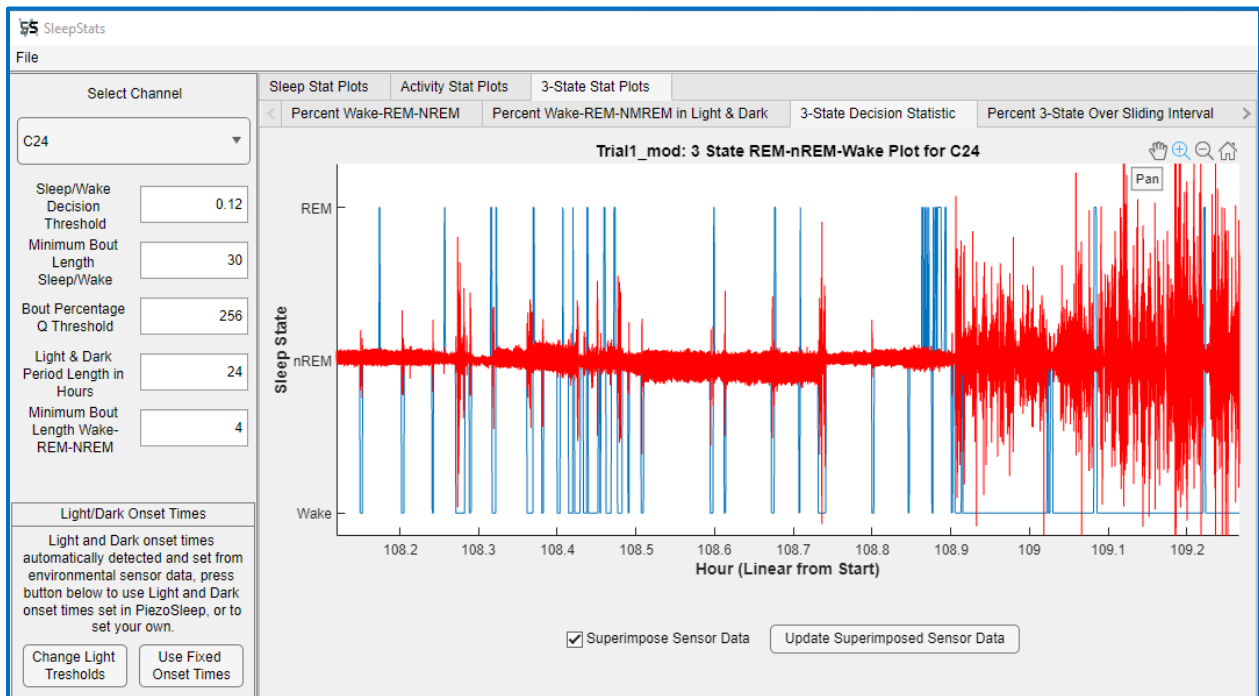
(b)

Fig 17: 3-state decisions with sensor signal overlay (a) full recording (b) zoomed in and updated to see 3-state transitions at a resolution where signal envelopes can be observed.

Figure 18 shows an example of zooming to a resolution where breathing changes associated with REM can be observed. In general, regular breathing rates are associated with NREM sleep behavior, and variation in breathing (frequency and amplitude) is associated with REM sleep behavior. As discussed in the performance analysis (**Appendix B**), this does not have a strong one-to-one correspondence with the human-detected brain state over short time intervals, unlike the changes between sleep and wake. The breathing changes tend to occur before and after a REM event, as well as within the REM event (the signal may become more regular for some intervals). See the descriptions of the validation experiments in Appendix B for more explanation.
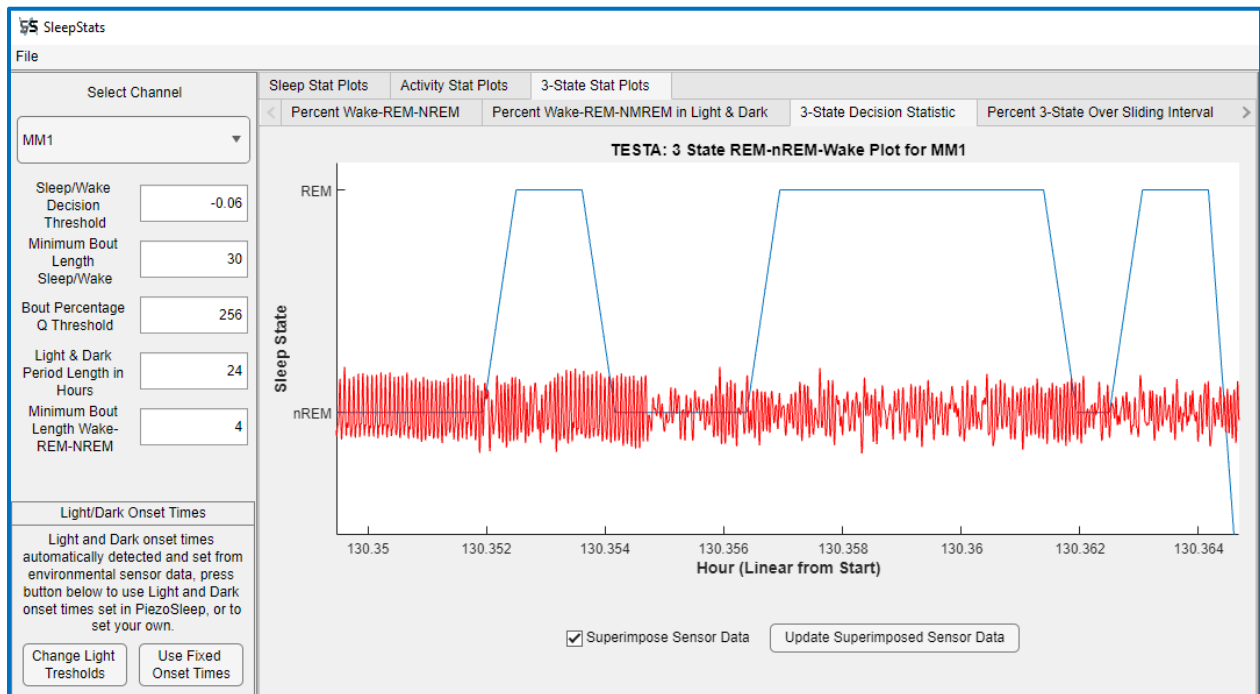
(a)
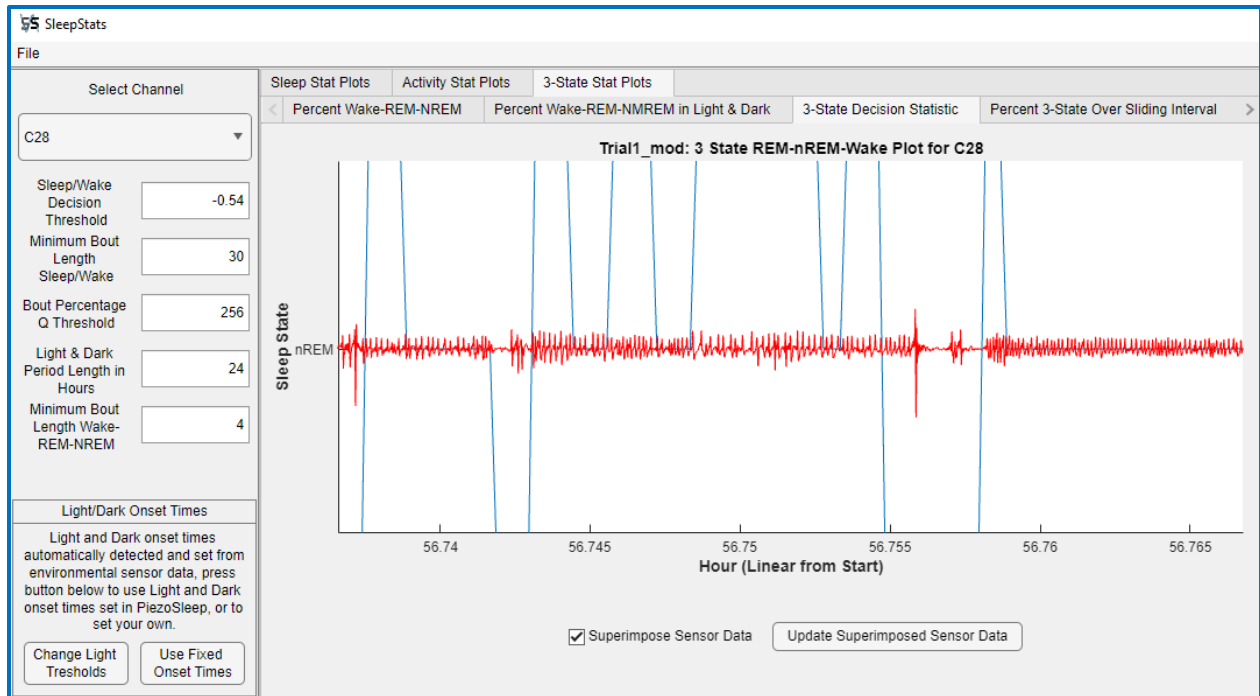
(b)

Fig 18: 3-state decisions with sensor signal overlay with a close-up on REM-like respiration.

Percent 3-State Over Sliding Interval

This is analogous to the percent state over a sliding interval for the 2-state case. The default interval for computing percent is 2 hours. The default increment for the percent computing interval is 0.2 hours (12 minutes). These settings can be changed in the text boxes on the bottom panel. Figure 19 shows an example of NREM and REM percentages over a 5-day recording. The y-axis remains fixed, so levels between the state and the animal are more easily observed. Since REM typically occurs much less frequently, the zoom feature should be used to reduce the y-axis to better see the changing dynamics over the time series (as used in Fig. 19b).
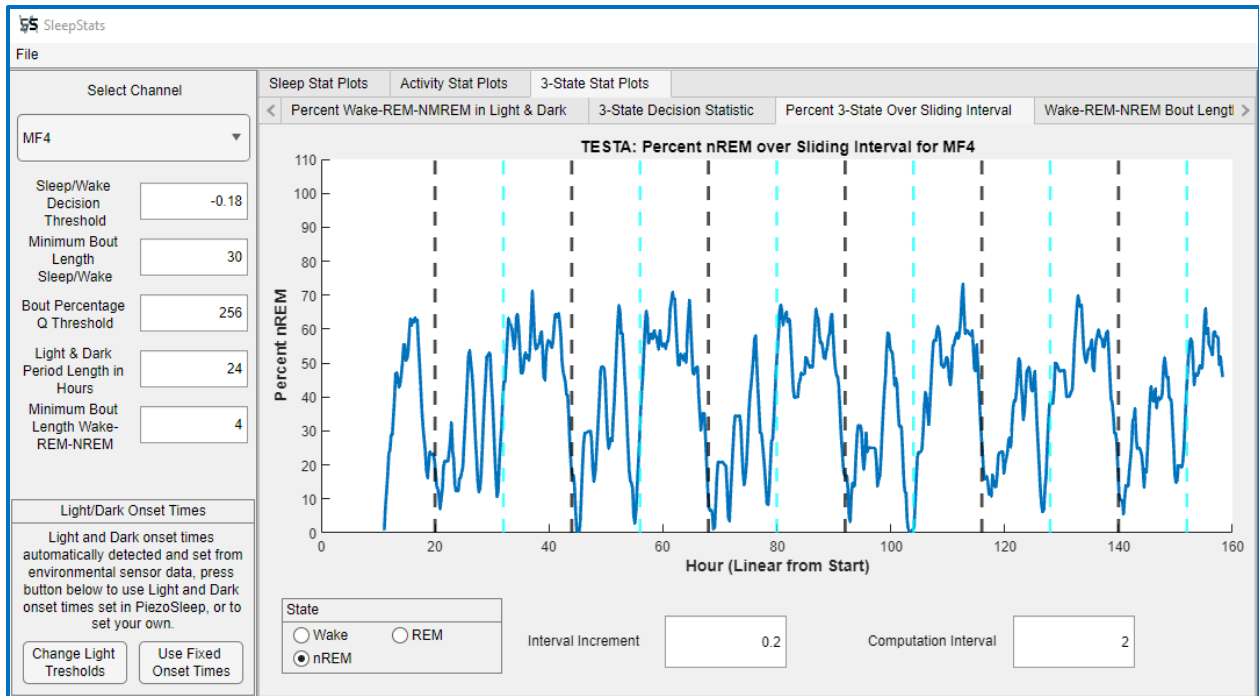
(a)
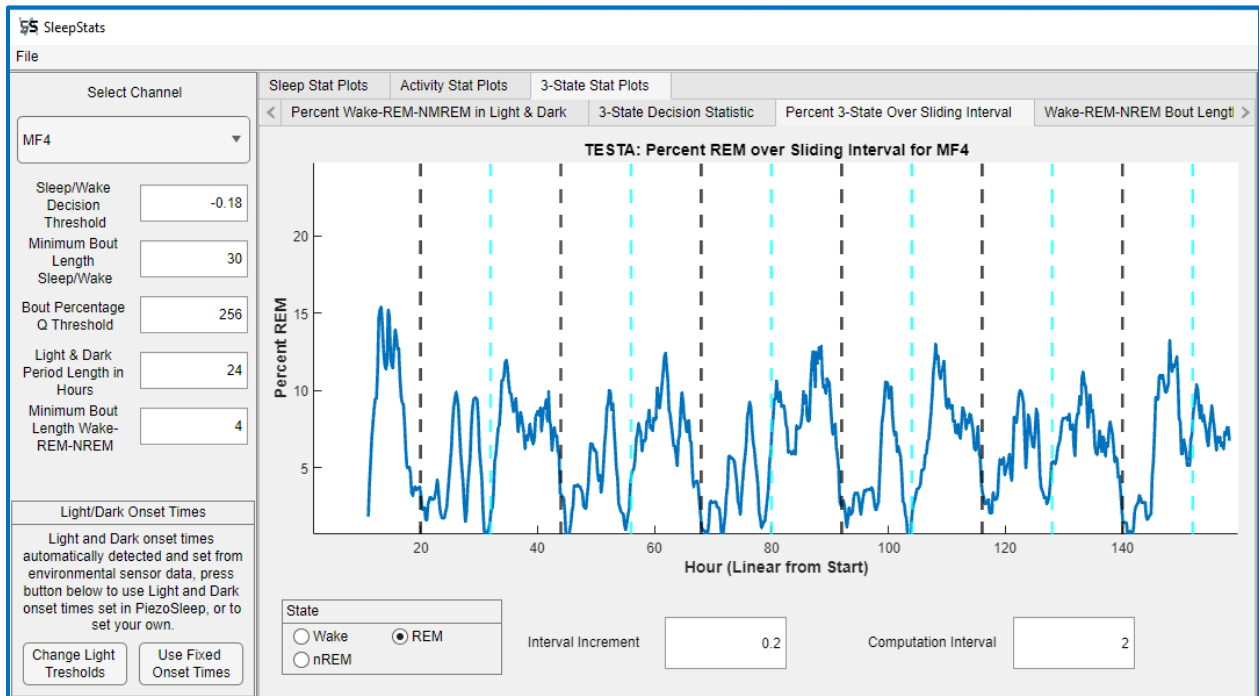
(b)

Fig 19: Percent state over sliding interval (a) NREM (b) REM zoomed in along the y-axis

Wake-REM-NREM Bout Length Histograms

The bout length histograms are computed as they are for the 2-state. In the 3-state case, the target behavior must be greater than 50% of the samples in the window, which may include

various combinations of the other 2 states. And since REM typically occurs far less often than wake or NREM, many of the REM events will be lost with a larger minimum bout length window like 30 seconds. Therefore, a separate box is provided for the minimum bout length for the 3-state analysis. Figure 20 shows an example of changing the minimum bout length from 6 to 30 seconds for the REM state. Note the difference in the y-axis when comparing the 2 figures. For the 30-second minimum bout length, many short REM bouts are missed since they do not fill up 50% of the 30-second interval. The plots show about 180 REM bouts in the 30 to 60-second range, whereas adding up the bouts over the 6 to 48-second interval (to approximate the 30-section interval for comparison) and are over 2000 REM bouts.
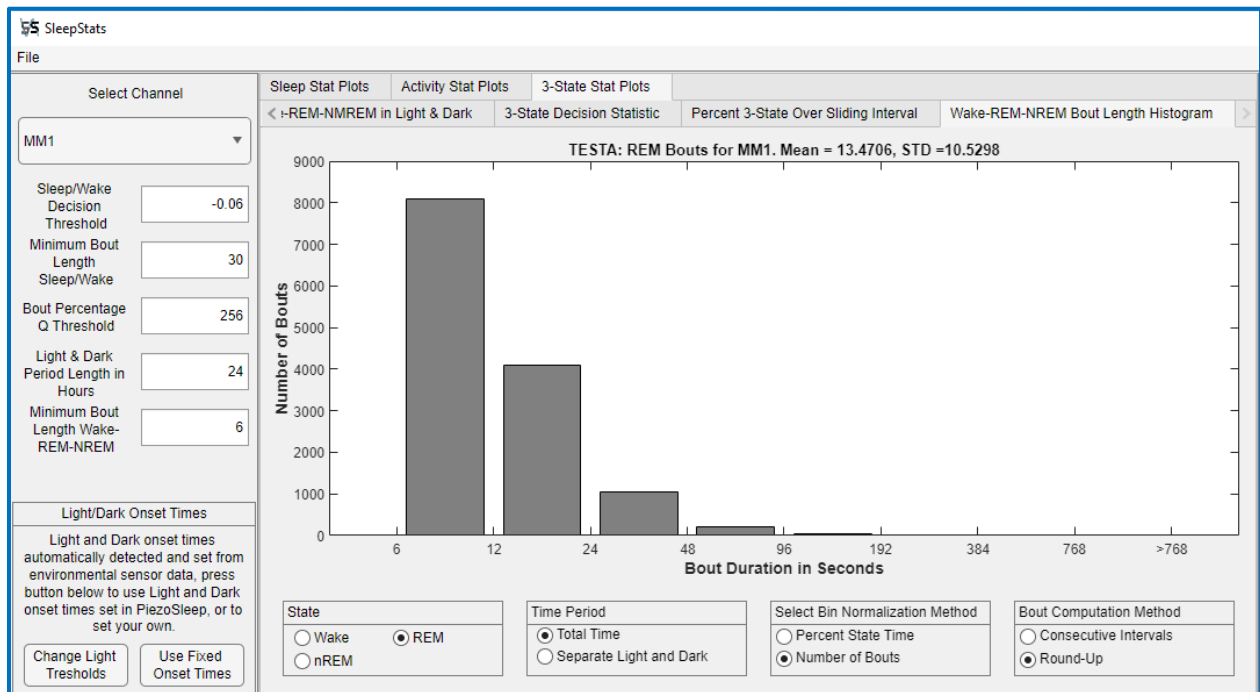
(a)
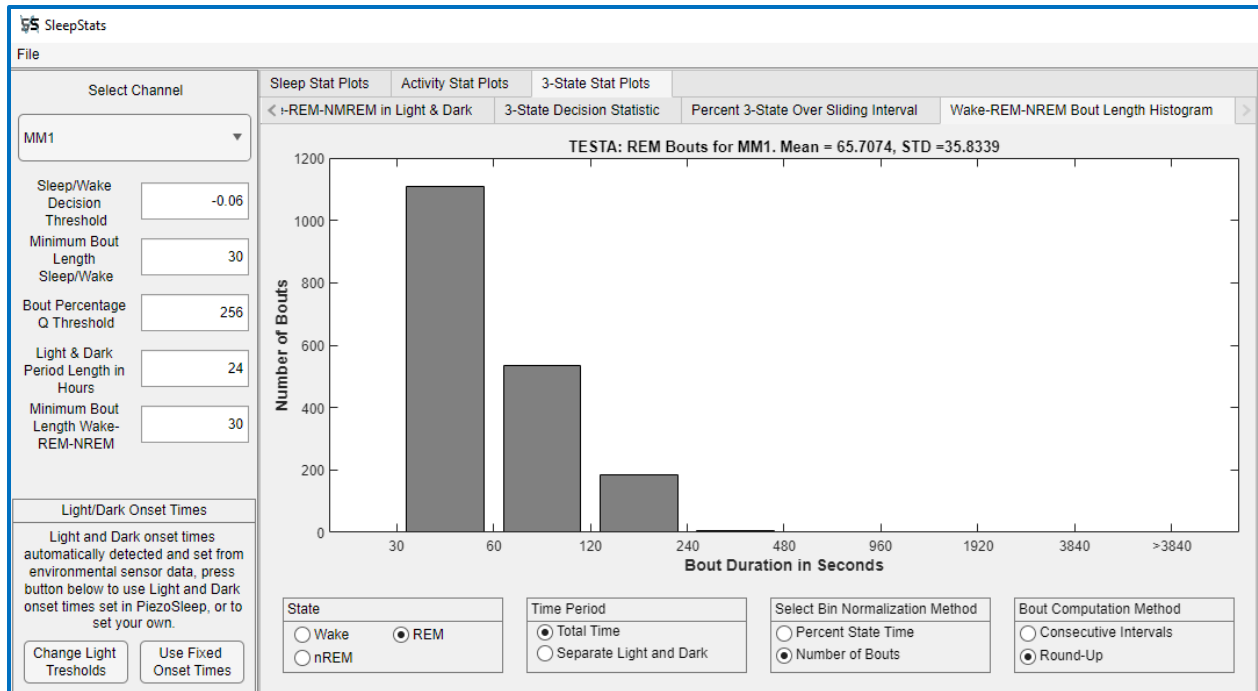
(b)

Fig 20: REM bout lengths showing the number of bouts for a minimum bout length of (a) 6 seconds (b) 30 seconds

Activity Stat Plots – **PRO Version Only**

The activity plots show animal activity patterns over the *PiezoSleep* recording. While changes in activity are readily seen when looking at the pressure sensor signal envelope, an activity level measure must be computed for use in quantitative analyses. The activity measured computed in SleepStats is proportional to the rate and relative force of motions over short time intervals (4 seconds). A calibration procedure is applied in computing activity levels to reduce variations, due to animal weight and sensor sensitivity differences. This procedure works best when at least 24 hours of data have been recorded to include a typical range of activities.

PiezoSleep computes and stores uncalibrated activity parameters in files ending in **_at.Feat** for rats or **_at.Featfb** for mice. Activity parameter time series are computed from the sensor signal samples taken over 4-second intervals that are incremented by 2 seconds (50% overlap between consecutive time intervals). In each interval, the samples are squared, averaged, and the square root is taken to create a root-mean-squared (RMS) value. The RMS values are multiplied by the time interval (4 seconds in this case) to create an energy-based value. This interval is incremented every 2 seconds to create the time series stored in the **_at.Feat(fb)** file.

The activity values are scaled and shifted in *SleepStats* so that the activity measure is 0 when the energy level is equal to or less than the median of activity values computed during sleep (motion from respiration). In addition, an activity level of 0 is assigned for all intervals detected as sleep in SleepStats. Typically, calibrated activity values less than 1 correspond to quiet wake

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and low activity states. Activity values near 2 correspond to slow head movements, slow locomotion, or gentle rearing. High levels of activity near 3 and above correspond to movements associated with more rapid locomotion, rearing, or grooming. The values are clipped at 6 to limit artifacts (like humans touching the cages) from significantly impacting computed activity statistics when averaged over larger intervals.

A graphical representation of activity patterns is presented in actogram plots, where daily pattern changes can be readily observed. Activity statistics can be exported to CSV files in 2 formats that are described in the export sections. For all computed/exported statistics, a larger interval time interval is selected to aggregate the activity measures (typically on the order of minutes) to create a more stable time series.

Actogram

The activity statistics plot consists of an actogram for observing activity patterns relative to the light and dark onset over the series of days recorded. The interval for computing average activity values that correspond to the activity bar heights is set in the textbox under the graphic as shown in Figure 21. Activity levels for the Actogram are clipped at 3, so the highest level of the plotted bars corresponds to level 3. A 10-minute default averaging interval is used for the levels plotted. Increasing or decreasing this interval typically does not change the pattern significantly, as the number of hours on each day line is 48. If the averaging interval is too big, the activity bars will have greater space between them, as seen in the trends demonstrated in Fig. 21a to 21c. Each row of the actogram includes part of the time from the following day. For example, the pattern after the second dark onset on Day 1 is repeated after the first dark onset in the Day 2 row. This way, continuity is better observed from one day to the next when looking horizontally, while pattern changes at a particular time of day are observed looking vertically. The actograms are made to observe changes over daily time scales, based on the density (blackness) of the vertical lines drawn in the axis, which are spaced for the 48-hour axis. So, zooming in on these figures is not of much value. If activity analyses are required on a finer interval, it is best to export these values on a desired time scale and create plots from the CSV file exports.
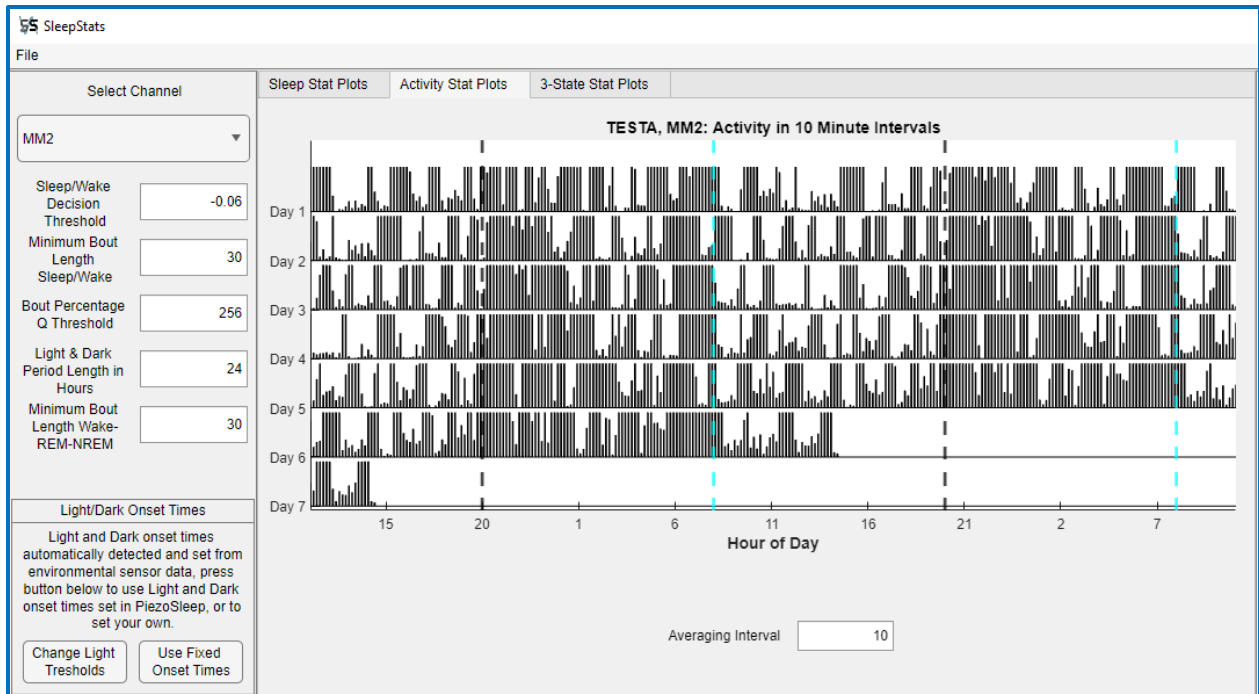
(a)
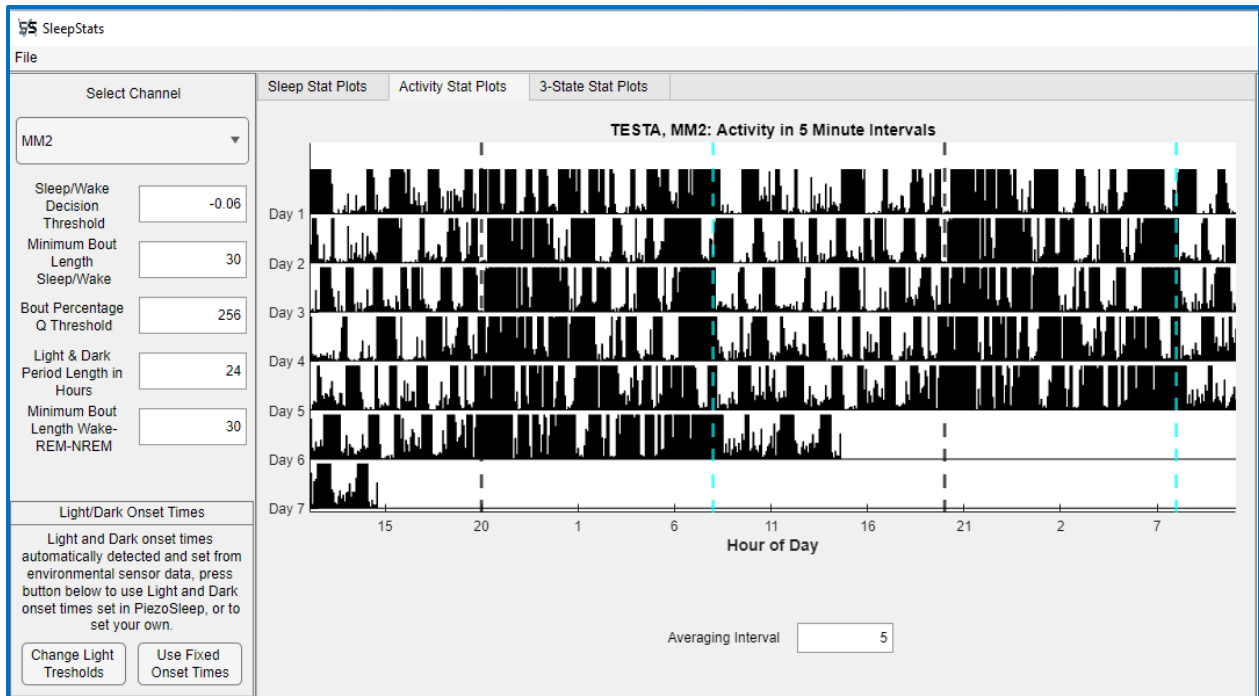
(b)

(c)

Fig 21: Actogram examples with a bin interval of (a) 10 minutes (b) 5 minutes, and (c) 15 minutes.

Right Panel - CSV Export

The right panel drop-down menu displays the export options available in SleepStats. The graphic displays provide an efficient way to observe the recorded data and sleep-wake patterns. However, this can limit the exploration and characterization of some experimental results. For custom analysis and precision in extracting portions of the data, *SleepStats* provides options for exporting data and parameters to comma-separated variable (CSV) files, which can be opened by other programs for further processing and analysis. Figure 22 shows the export file options in the drop-down menu in the right panel. The options shown in this figure are all available for the **Pro** version. The **Base** and **Lite** versions will show a subset of these.
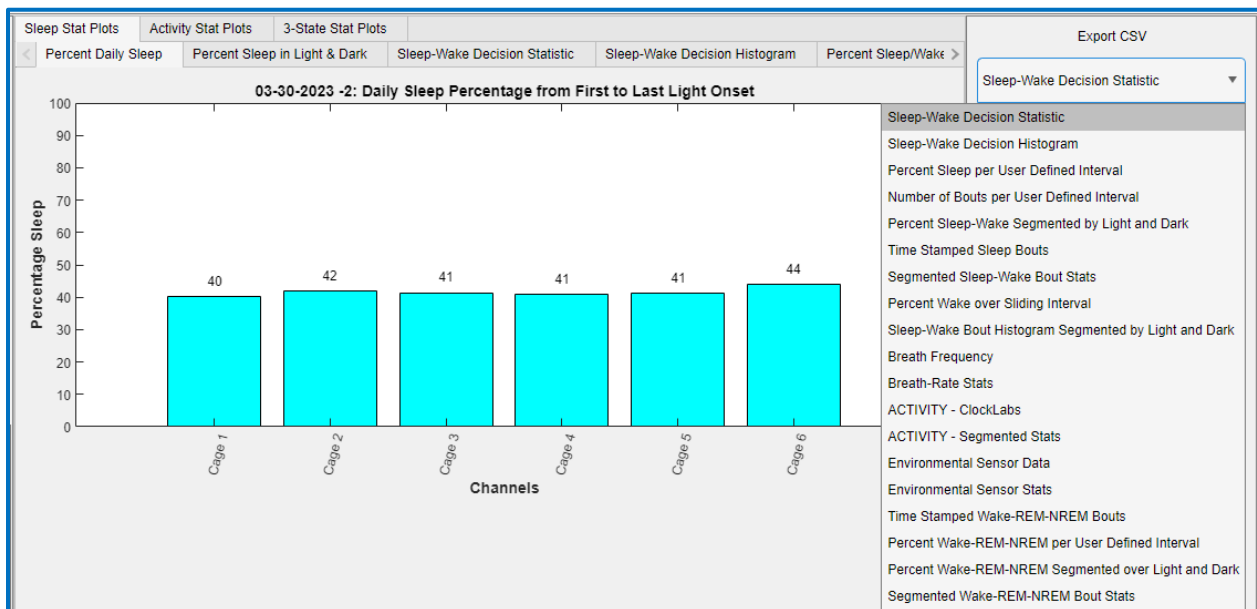
Fig 22: CSV Export panel with options shown in the drop-down menu

Sleep-Wake Statistic Exports

The following export options discussed in this section are for the 2-state sleep-wake results.

Sleep-Wake Decision Statistic

This export generates a CSV file with the sleep-wake decision statistic. Once the export button is pressed, a pop-up menu will appear, allowing the user to adjust the start and end times for the export, as well as the base CSV filename. This option is not recommended for long recordings or exported segments. It is usually best to compute statistics over larger time intervals, as some of the other options do. This export may take a long time, depending on the size of the export. For example, for 20 hours of data, 36,000 lines of data will be written for each channel. The export has a timestamp column, using the same time axis units as in the *Sleep-Wake Decision Statistic* time-series plots.

Sleep-Wake Decision Histogram

This generates a CSV file with the data from the histograms shown in the *Sleep-Wake Decision Histogram* plot tab. The first row is the channel ID, the second identifies each column as the left bin edge and the number of occurrences within that bin. An example of the output from this export is shown in Figure 23. The number of values between the bin edges are all values greater than or equal to the lower edge and less than the upper edge. Except for the last bin, which also includes equal to the upper edge. The bin edges listed in the exported spreadsheet correspond to the lower edges of the bin interval, the upper edge would be the edge listed in the next row.

Cage 1		Cage 2		Cage 3		Cage 4		Cage 5		Cage 6	
Bin Edges	Number in Bin	Bin Edges	Number in Bin	Bin Edges	Number in Bin	Bin Edges	Number in Bin	Bin Edges	Number in Bin	Bin Edges	Number in Bin
-8	4	-8	35	-8	33	-8	19	-8	29	-8	49
-7.89	2	-7.86	28	-7.86	22	-7.85	30	-7.89	0	-7.88	29
-7.79	2	-7.72	24	-7.73	28	-7.7	26	-7.78	5	-7.76	18
-7.68	0	-7.58	27	-7.59	26	-7.54	43	-7.67	6	-7.64	30
-7.57	6	-7.44	31	-7.45	62	-7.39	63	-7.56	2	-7.52	30
-7.47	2	-7.3	58	-7.32	49	-7.24	78	-7.45	3	-7.41	37
-7.36	2	-7.15	83	-7.18	76	-7.09	131	-7.33	3	-7.29	44
-7.25	2	-7.01	87	-7.04	121	-6.94	136	-7.22	4	-7.17	67
-7.14	1	-6.87	122	-6.9	126	-6.78	237	-7.11	2	-7.05	64
-7.04	2	-6.73	152	-6.77	159	-6.63	269	-7	1	-6.93	90
-6.93	2	-6.59	169	-6.63	175	-6.48	350	-6.89	4	-6.81	104
-6.82	1	-6.45	239	-6.49	221	-6.33	415	-6.78	3	-6.69	137
-6.72	1	-6.31	291	-6.36	304	-6.18	545	-6.67	9	-6.57	146
-6.61	4	-6.17	344	-6.22	328	-6.02	694	-6.56	3	-6.45	221
-6.5	0	-6.03	421	-6.08	411	-5.87	857	-6.45	5	-6.33	214
-6.4	2	-5.89	485	-5.95	505	-5.72	1002	-6.34	7	-6.22	276
-6.29	1	-5.74	580	-5.81	580	-5.57	1167	-6.22	4	-6.1	292
-6.18	2	-5.6	707	-5.67	622	-5.42	1316	-6.11	13	-5.98	391
-6.07	7	-5.46	765	-5.53	731	-5.26	1495	-6	5	-5.86	417
-5.97	3	-5.32	949	-5.4	772	-5.11	1631	-5.89	9	-5.74	486
-5.86	3	-5.18	1117	-5.26	866	-4.96	1811	-5.78	17	-5.62	571
-5.75	5	-5.04	1242	-5.12	903	-4.81	1946	-5.67	23	-5.5	620
-5.65	1	-4.9	1355	-4.99	984	-4.66	1983	-5.56	13	-5.38	717
-5.54	10	-4.76	1525	-4.85	1105	-4.5	2118	-5.45	25	-5.26	832
-5.43	2	-4.62	1547	-4.71	1179	-4.35	2027	-5.34	38	-5.14	875
-5.33	1	-4.47	1655	-4.57	1192	-4.2	2115	-5.23	37	-5.03	949
-5.22	1	-4.33	1725	-4.44	1220	-4.05	2225	-5.11	39	-4.91	953

Fig 23: Example of a Sleep-Wake Decision Histogram CSV export.

Percent Sleep-Wake per User-Defined Interval

This export generates a CSV file with percent sleep computed over a user-defined interval in minutes, with a default value of 60 minutes. The first column of the export consists of time stamps corresponding to a sequence of start times for each interval. Once the export button is pressed, an options menu window will appear with the start and stop time of the complete file, and the last saved or default user-defined interval. An example of this window is shown in Figure 24.

These text boxes can be edited to change the time range for the export, the interval for the percent sleep or wake calculation, as well as the base file name for the output file.

In the CSV file, the first row contains the date the experiment began, followed by the channel IDs. The time stamps begin with the start time from the pop-up menu and represent the actual

time of day in a 24-hour format. If time is selected outside the recording range, it will be coerced to the beginning or end of the file.

Number of Bouts per User-Defined Interval

This export functions similarly to the Percent Sleep-Wake per User Defined Interval export, except it exports the number of sleep or wake bouts detected within the interval.

Percent Sleep-Wake Segmented by Light and Dark

This export is analogous to the *Percent Sleep in Light & Dark* plot. For this export, however, it exports sleep or wake percentage values for each day in the dark and light cycles (the plot average over all days). It also includes partial light or dark periods at the end or beginning of the recording. The first light or dark cycle is labeled as day 0. Day 1 starts with the first light onset.

Fig 24: Menu that allows for changing the: Start Time, End Time, Interval (in minutes), and the CSV output Base Filename.

Time-Stamped Sleep Bouts

This export brings up the same pop-up menu as in the *Sleep-Wake Decision Statistic* export option, which allows the user to select the start and end times within the recording to export, as well as the base CSV file name. This exports a separate CSV for each channel with the following format: ***base-nameSB_ChanID.csv***. In each CSV file, there are three columns: the time stamp which contains the time and date, linear time, which corresponds to the time "axis" on many of the time-series plots, and the length of the bout at that time stamp. Since the starting bout times for each animal is different, as well as the number of bouts, the time-stamped series for each animal is exported to its own CSV file.
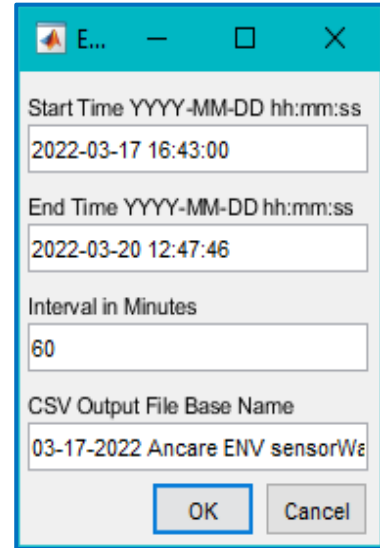
Segmented Bout Stats

This export works like the *Percent Sleep per User Defined Interval*. It exports several bout statistic files that describe sleep or wake behavior. These statistics include the histogram bin values, mean, median, standard deviation, 10th percentile, 90th percentile, and Qth percentile (the percentage of bouts greater than a user-defined threshold, default is 256 seconds). The bout lengths are all in units of seconds.

Since not all these statistics may be relevant to a study, an options panel is presented under the Export CSV drop-down menu, as shown in Figure 25. This allows the user to select the statistic files to export. For each item selected, a spreadsheet is generated with the corresponding statistics for each time interval and channel. The only exception to this is the histogram export, which outputs histogram values over the entire time range of the export. When the export button is pressed, a window (as in Fig. 24) appears, allowing the user to change the time range, the base filename of the CSV output, and the size of the intervals for the stats to be calculated.
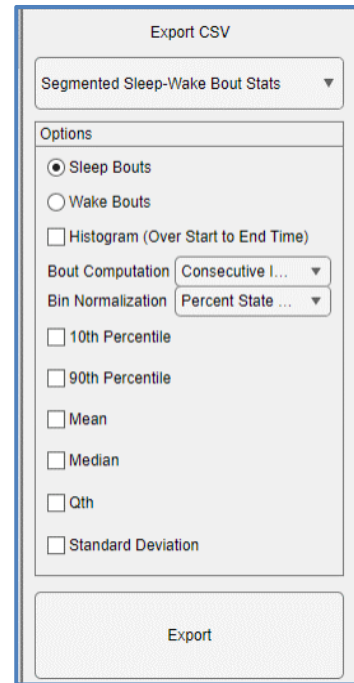
Fig 25: Options for exporting Segmented Bout Stats.

The statistics in the generated CSV files are identified by strings appended to the base CSV filename. The strings appended to the files names of the different statistics exported are shown in the following table.

Sleep Bout Statistic	Units	Appended String for Sleep	Appended String for Wake
Histogram	Percent Time	_histogramSB	_histogramWB
10th Percentile	Seconds	_10pctileSB	_10pctileWB
90th Percentile	Seconds	_90pctileSB	_90pctileWB
Mean	Seconds	_meanSB	_meanWB
Median	Seconds	_medianSB	_medianWB
Qth (Percent Greater than Threshold Q)	Seconds	_QthSB	_QthWB
Standard Deviation	Seconds	_StdSB	_StdWB

If an interval does not include at least one of the selected bouts, a NaN (not a number) is output. A zero is not appropriate in this case, since the length is being computed and not the number of bouts. If there are no bouts detected, that does not mean a length of 0. The NaN represents that a length could not be computed because no bout existed to measure. Many

spreadsheet and mathematical programs have functions that will automatically ignore NaN values when computing statistics, such as mean and standard deviations.

Percent Sleep/Wake Over Sliding Interval

This export option saves the values from the *Percent Sleep/Wake Over Sliding Interval* plot to a CSV file, using the same increment and interval settings from the plot. Parameters for this export will be the same as they were for the graphic plots; however, the menu in Fig. 26 provides the opportunity to change these parameters. Changing the values in the options panel for the export changes them in the plot as well. The same goes for changing the values within the plot tab. These number fields are linked in the program.
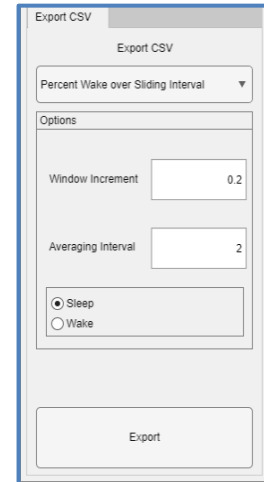
Sleep-Wake Bout Histogram Segmented by Light and Dark

This exports values that are presented in the *Sleep/Wake Bout Length Histogram* plots when the *Separate Light and Dark* option is selected. The spreadsheet output is arranged as a series of tables horizontally,

Fig 26: Percent Wake Over Sliding Interval export options.

where each table denotes a light or dark period. The columns within each table are the lower limit of that bin, with the last bin's upper edge going to infinity, and the rows can be either the number of bouts in each bin or the percent time spent in the bout range (depending on what was selected on the export menu). The table below shows an example of two periods in a CSV file exported by this function with percent state time normalization (adds up to 100% over each row, may be off a little due to rounding).

	Day 0 Dark								Day 1 Light							
Lower Bin	30	60	120	240	480	960	1920	3840	30	60	120	240	480	960	1920	3840
Cage 1	28.86	17.13	31.49	21.51	0	0	0	0	4.24	4.29	8.07	6.36	5.89	14.93	22.9	33.14
Cage 2	0.55	0	0	4.56	23.62	10.49	60.79	0	0.75	0.37	0.69	1.3	4.7	7.55	43.79	40.85
Cage 3	9.16	5.84	8.68	2.46	0	0	73.87	0	2.13	3.95	2.79	7.15	12.56	11.27	60.15	0
Cage 4	2.98	1.86	3.49	3.4	8.6	36.84	0	42.83	1.2	0.41	0.56	4.34	2.19	21.3	70	0
Cage 5	12.79	14.15	17.43	13.17	11.31	31.14	0	0	3.88	4.07	7.6	7.51	6.39	15.53	23.52	31.41
Cage 6	4.09	3.64	3	19.38	17.89	21.02	30.83	0	1.43	2.54	2.5	11.28	16.58	16.33	35.1	14.25

Breathing Data Export

Reliable breath rate estimates require a good signal-to-noise ratio. Therefore, for mice, it is only recommended for film sensor system recordings in low-noise environments. The Adapt-A-Base signals tend to have lower signal-to-noise ratios as compared to the film-based sensors, and therefore, breath rate exports from data collected on AAB are not recommended for mice. Files ending with strings *_br.Featfb* for mice or *_br.Feat* for rats should be in the folder along with the feature vector file. These files contain estimates for respiration rates computed over local 4-second intervals with a 50% overlap (2-second increments) that can be exported. The next 2 options explain these exports.

This export operates in the same way as the *Sleep-Wake Decision Statistic* export, except it outputs Breath Frequency data. This export could take some time as it exports values every 2 seconds. Note that the breath frequency calculations are set to NaN when the breath frequency is either out of a reasonable range, or while the animal is too active, and a respiration signal cannot be detected. Therefore, there may be a lot of NaNs during the wake states in the exported CSV file.

Breath-Rate Stats

This export works similarly to *Segmented Bout Stats*, except instead of bout length statistics, it exports breath rate statistics over a user-defined interval. This can export mean, median, and standard deviation breath rates over an interval, depending on which checkboxes were selected before the export button was pressed. Once the export button is pressed, a window similar to the one shown in Fig. 24 will pop up, allowing the user to set the start and end times of the export, the interval over which the statistics are calculated, and the base CSV filename used for the export. Each statistic selected in the options panel is then exported to separate CSV files, with their file names being the base CSV filename with BreathStats and _mean, _median, and _std appended to it for mean, median, and standard deviation exports respectively. NaN values are ignored in the calculations.

Activity Exports – **PRO Version Only**

The following exports use the activity calculations written to the activity feature file ending in *_at.Featfb* for mice and *_at.Feat* for rats.

ACTIVITY – ClockLabs

This export generates activity values (from the actogram) into a file that is compatible with the **ClockLabs** program from Actimetrics (Wilmette, IL, USA). The **ClockLabs** program computes statistics from activity patterns that are typically used in circadian studies. Activity measures for **ClockLabs** are based on wheel running patterns (the number of revolutions per minute being proportional to activity levels). The numbers from the **PiezoSleep** system are not as precisely defined but relate to activity over a larger range of behaviors (see *Activity Stats Plots* section), in that any motion is captured and converted to an energy-based value. This value is proportional to the density of the motion (i.e., how often a motion is made within a given interval) and the force of the motion. The calibration described in the introduction of the *Activity Stats Plots* section scales the values so they can be interpreted by the **ClockLabs** program (low activity to high activity).

This export selection generates a prompt like the one shown in Fig. 24. The user can select start and end times, as well as trim data off at the beginning and end, which is useful for synchronizing the intervals to times of other events. The user can select an interval as low as 1 minute. The exported file name typed into the textbox will be appended to the channel label and the base file name of the recording. After selecting *OK* in the prompt window, a file for

every channel will be created and stored in the same directory as the feature vector file. Each file name begins with the cage number and has an **awd** extension.

The header for the **ClockLabs** file has the channel ID, the start date and time to the nearest minute, and the interval between data points. The light and dark onset times are not transferred through this file when opened in **ClockLabs**, so these times will need to be added once the file is opened with **ClockLabs**. Since **ClockLabs** provides an option to bin data over specified time intervals, it is best to export with an average interval of 1 minute. This will provide more options once ported into **ClockLabs** to use larger intervals if desired.

ACTIVITY – Segmented Stats

This export works similarly to *Segmented Bout Stats*, except instead of bout length statistics, it exports activity level statistics over a user-defined interval. This can export mean, median, standard deviation, minimum, and maximum activity levels over an interval, depending on the checkboxes selected before the export button was pressed. Once the export button is pressed, a window similar to the one shown in Fig. 24 will pop up, allowing the user to set the start and end times of the export, the interval over which the stats are calculated, and the base CSV filename used for the export. Each statistic selected in the options panel is then exported to separate CSV files, with their filenames being the base CSV filename with ActStats and _mean, _median, _std, _max, and _min appended to it for mean, median, standard deviation, minimum, and maximum exports, respectively.

Environmental Sensor Exports

The following export options create CSV files from data recorded by the environmental sensor. These data are saved in the environmental sensor data file with the extension **Env** in the same folder as the feature vector file.

Environmental Sensor Data

This export saves the environmental sensor data to individual files for each data type: Light Levels (Lux), Pressure (in Hg), Relative Humidity (%), and Temperature (°F). This is exported in the same way as the Decision Statistic Export and the Breath Rate Export, in that it exports large amounts of data, and the user can select the start and end times to export, as well as the base filename for the export.

Environmental Sensor Stats

For more efficient exports, statistics from the environmental data can be extracted. These files are typically smaller as they describe the conditions over larger time intervals. This can export average and median environmental sensor data over an interval. Once the export button is pressed, a window like the one shown in Fig. 24 will pop up, allowing the user to set the start and end times of the export, the interval over which the stats are calculated, and the base CSV filename used for the export. Each statistic selected in the options panel is then exported to separate CSV files, with their filenames being the base CSV filename with the string *EnvironmentalSensorStats* and *_average and/or _median* appended to it for average and median exports, respectively.

3-state (Wake-REM-NREM) Exports – **PRO Version Only**

The following exports use the 3-state classifications saved in a 3-state feature file ending in _3s.wss.

Time Stamped Wake-REM-NREM Bouts

This is analogous to the 2-state export, except any of the 3 states can be selected.

Percent Wake-REM-NREM per User-Defined Interval

This is analogous to the 2-state export, except any of the 3 states can be selected.

Percent Wake-REM-NREM Segmented over Light and Dark

This is analogous to the 2-state export, except any of the 3 states can be selected.

Segmented Wake-REM-NREM Bout Stats

This is analogous to the 2-state export, except any of the 3 states can be selected.

Appendix A - Manual Threshold Settings for Sleep-Wake Decisions

The characterization of sleep and wake behavior from the animal pressure signals involves **signal amplitude, quasi-periodicities,** and **regularity/consistency** of the signal amplitudes. Relatively low amplitudes imply smaller/weaker movements that are typical for sleep and rest, and small amplitudes with signal regularity (quasi-periodic signal with consistent amplitudes) imply regular breathing with no conscious activity. The latter pattern is critical for distinguishing sleep from quiet wake or rest. These signal features are used to model sleep-like behavior. A small interval (four to eight seconds) is used to extract signal features from the pressure waveform and are combined to form a single number, referred to as the decision statistic. This is performed every two seconds with overlapping windows.

In a typical case for a recording that covers thousands of decision statistics, the animal has been awake and asleep (a total of 43,200, two-second intervals in a 24-hour period). A histogram of the decision statistics will typically be bimodal as shown below in *Fig A 1*, where it is assumed the decision statistics are clustering around these two dominant behaviors.
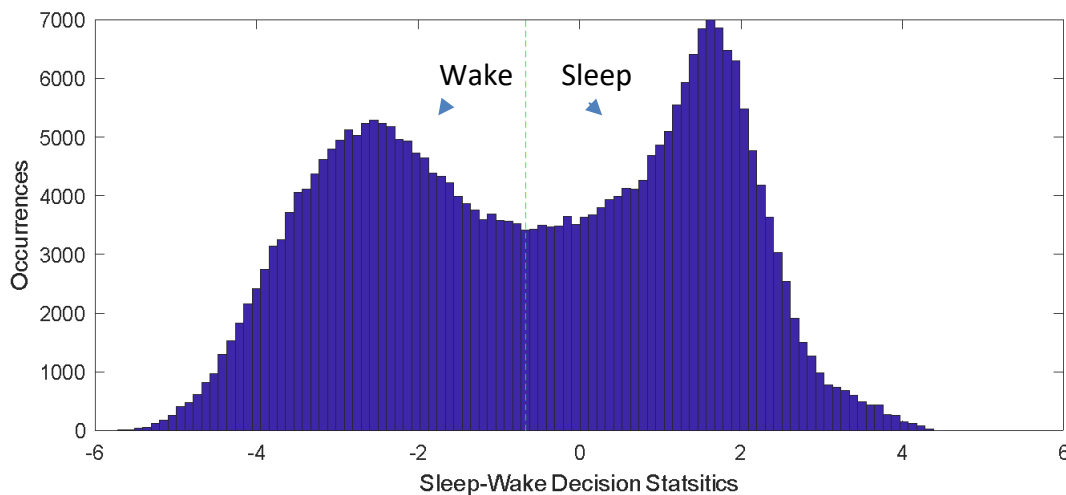

Fig A 1: An example of a good bimodal distribution of decision statistics.

The vertical green line is the threshold between sleep and wake placement. It will typically fall in the saddle point between the two modes. The modal point towards the positive side represents clusters of the decision statistics resulting from sleep behaviors, while the modal point towards the negative results from wake behavior. In many cases, the wake behavior is more varied, and the peak may not be as distinct as in the sleep behavior, as shown in Fig A 2 below:
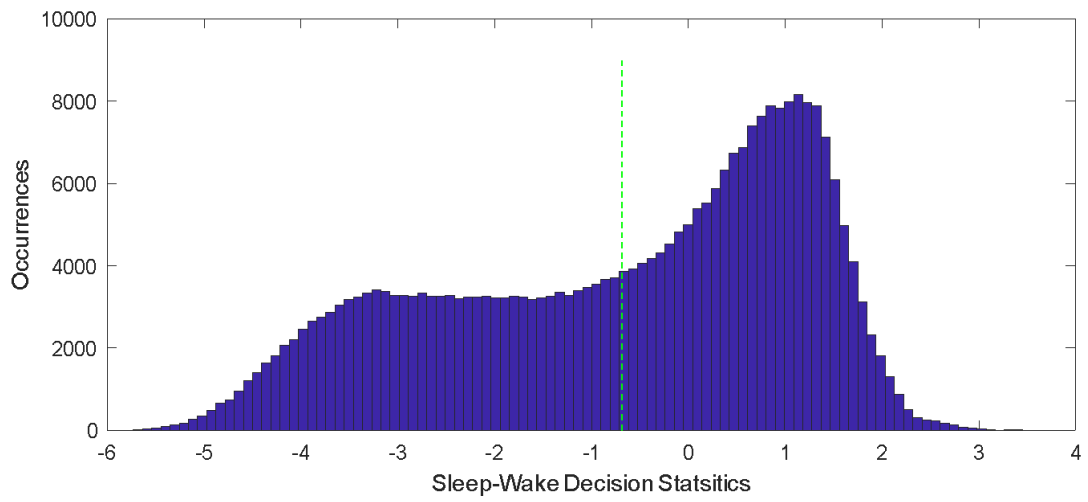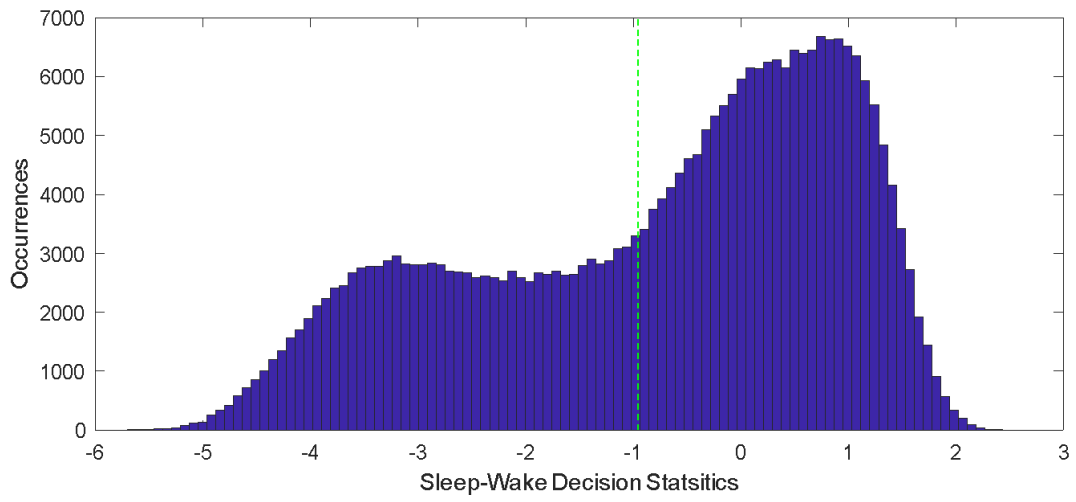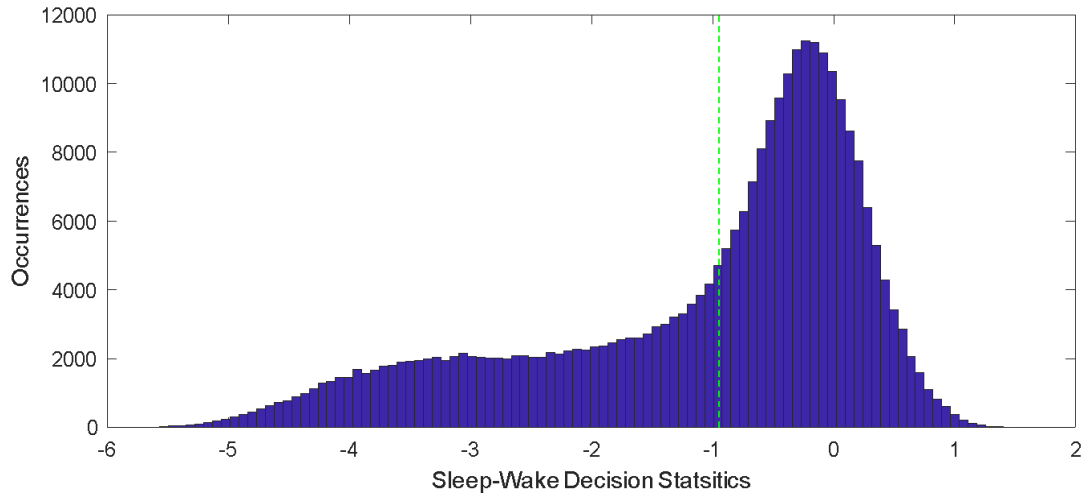
Fig A 2: Examples of high variance in the wake side of a bimodal distribution.

The histograms above suggest the algorithm worked properly to separate the sleep and wake behaviors, assuming both behaviors were present during the recording. In these cases, which should be most of the time, the threshold will not need to be adjusted.

The algorithm relies on relative differences over the course of a recording to calibrate features and classify behaviors. Sometimes decision statistics can be shifted higher or lower for different animals, depending on their weight, breathing patterns, and levels of activity or restlessness. The level of noise in the environment can also shift the decision statistic values, especially when it degrades the periodic pattern for low-level sleep signals. While the decision algorithm targets a threshold of zero for a typical animal, an adaptive threshold is applied to accommodate for the variations in animal and recording environments. The automatic algorithm attempts to find a threshold that separates the decision statistic, such that the sum of the resulting variances from both sides is minimized. This works in almost all cases of weak signals, larger noise levels, and unusual, or illness-like behaviors, the bimodal pattern can degrade. This can cause the algorithm to set the threshold in an unusual or wrong place. Examples of high noise recordings are shown in Fig A 3, where the last image is from an empty cage. Since no animal was changing its behavior, just noise, it has a string unimodal and symmetric shape.
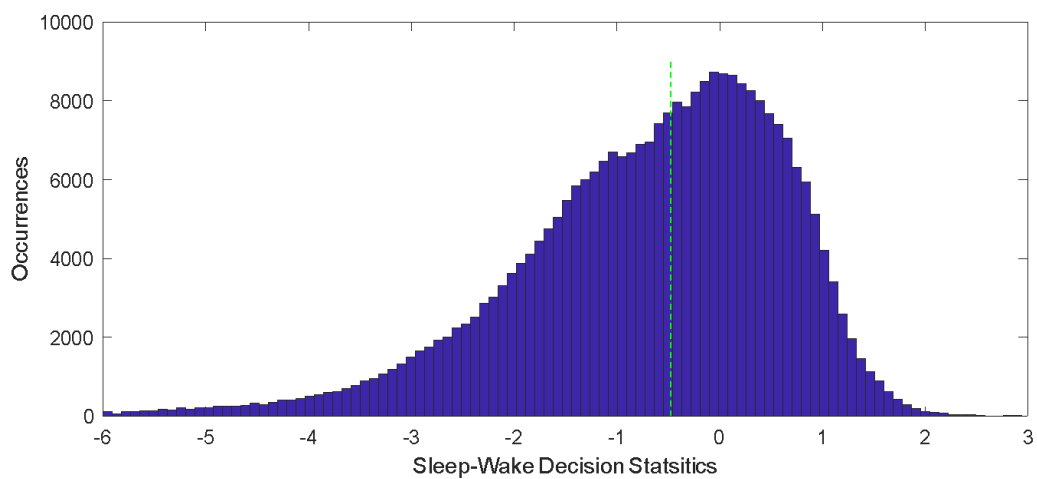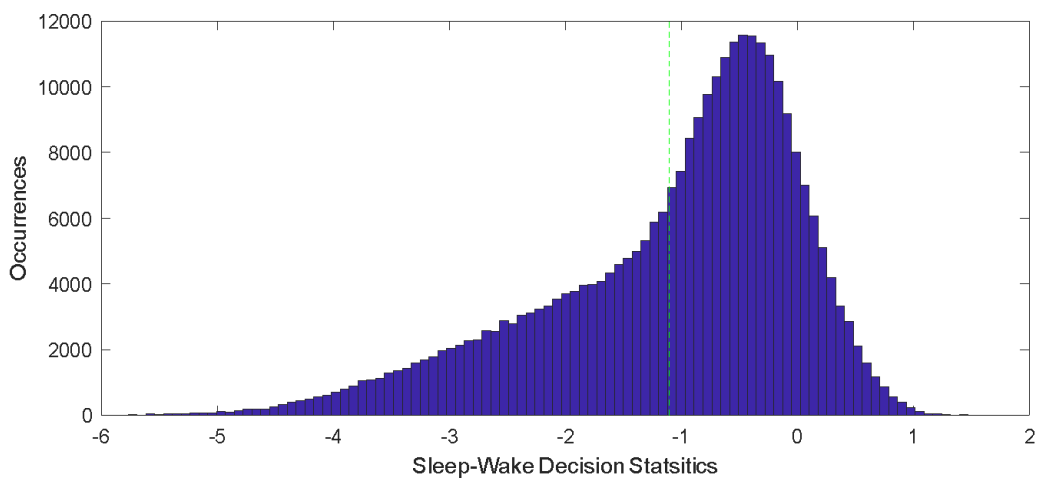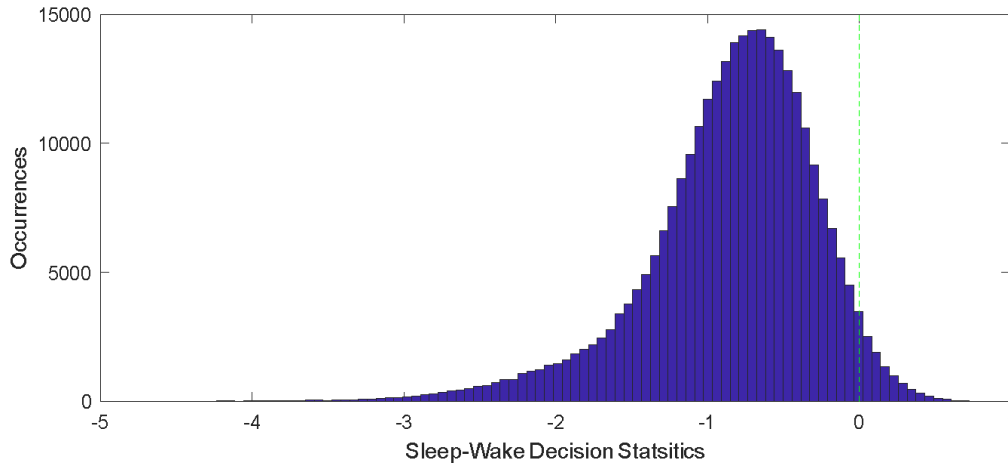
Fig A 3: Examples of high noise statistics, the last is an empty cage (showing only noise)

When an extreme or unusual pattern is observed, such as an odd-shaped decision statistic histogram or sleep percentages are too high or too low, the pressure signal should be inspected for integrity. If a BAS signal is observed, for example, if typical patterns of wake or sleep activity are not observed, or amplitudes are too low or high overall, the results should not be trusted.

It may be possible to manually set the threshold through inspection of the histogram and time series of the decision statistic if typical sleep and wake patterns can be seen in the pressure signal. This adjustment involves a level of subjectivity as to what pressure signals are sleep or wake-like.

Below, in Fig A 4, is an example of a case that showed high levels of sleep, around 80%-90%. Note the decision statistics are shifted high, towards sleep, and the histogram is very narrow. This was a channel where the signal was consistently a low amplitude, as if there was a faulty sensor, amp, or connection, or there could have been an issue with the animal that resulted in limited movement.
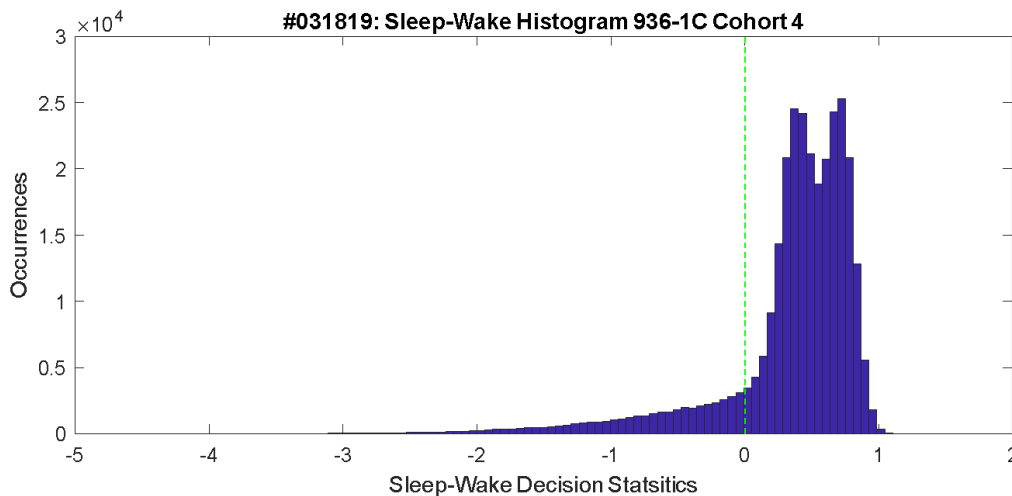

Fig A 4: Example from a weaker-than-usual signal.

To provide more insight into what is happening, use the third tab under *Sleep Stat Plots*, to see the *Sleep-Wake Decision Statistic*, for this channel. The plot should look something like Fig A 5 below.
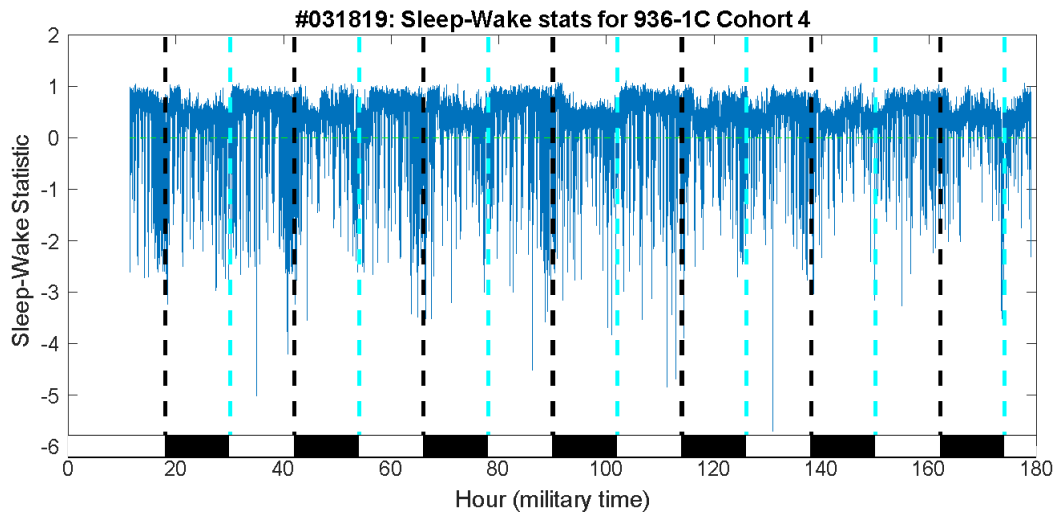

Fig A 5: Time series decision statistic for the same channel as Fig A 4.

In the decision statistic time series, the green horizontal line is the threshold for the sleep and wake decisions. The histograms would result from projecting the data into the y-axis representing the decision statistic values. Note, a mile trend is observed with a dip in values during the dark phase, when wake-like behavior would be expected, and higher values in the light phase, where sleep-like behavior is expected. This suggests the threshold should be put between the clusters. The figure above is zoomed in to a few hours around a dark-to-light transition (light-to-dark works as well), as in *Fig A 6* below:
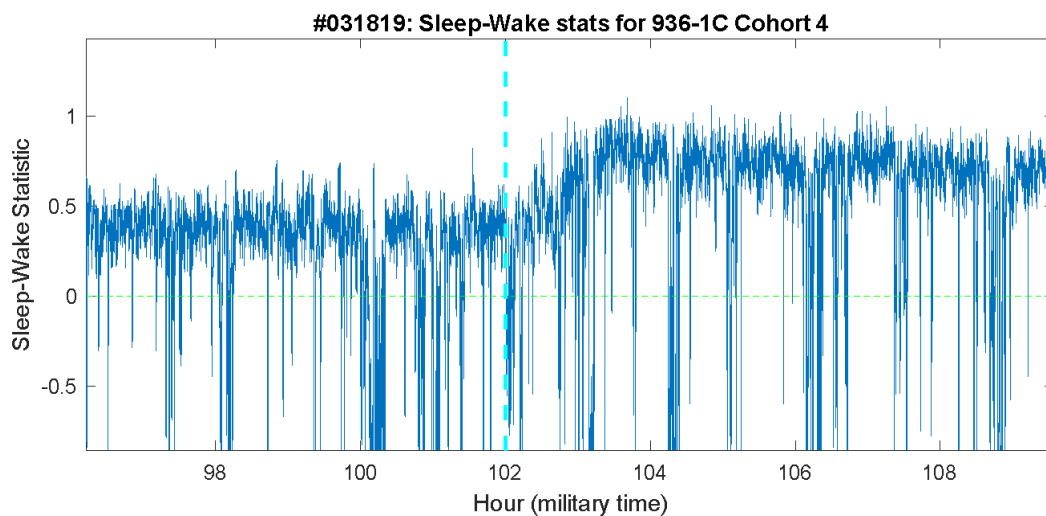

Fig A 6: Zoomed in statistic plot from Fig A 5

50

Now, the **Superimpose Sensor Data** checkbox can be checked, which overlays the original pressure signal (*Fig A 7*), and the source of the problem is observed. The amplitudes for activity patterns are relatively small, usually, activity is at least 40 mV, and the signal is much smaller than what is typically recorded. This could be the result of faulty hardware or a weak animal. The low-level sensor data can be observed closer since on that small of a scale the sleep and wake patterns can be observed through higher and lower activity, though the signal is more corrupted by system noise than a higher amplitude signal would.
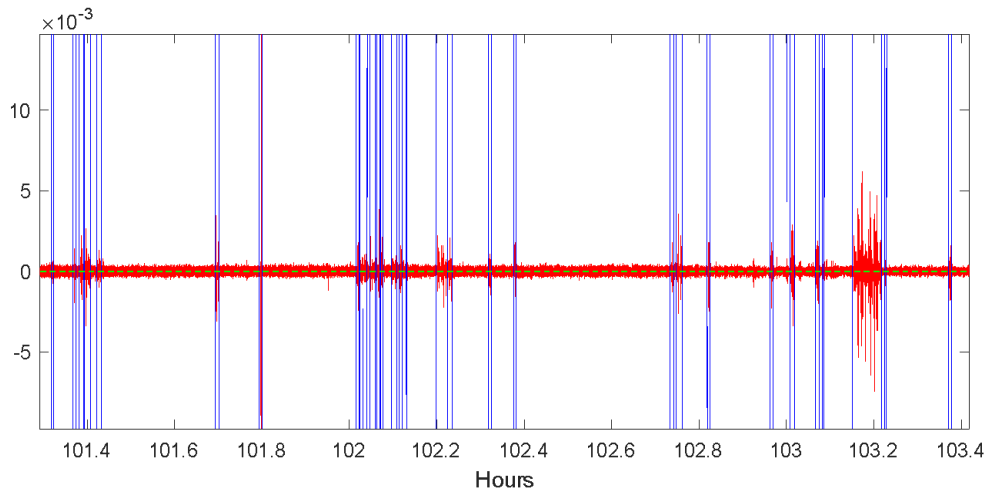

Fig A 7: Decision statistic with sensor signal superimposed.

For this case, a threshold around 0.4 or 0.5 would be appropriate. The histogram from Fig A 4 can be zoomed in on to try and find a more precise threshold setting. To change the decision threshold for this channel, simply change the **Sleep/Wake Decision Threshold** value in the left panel.

Below in Fig A 8, is another example of an outlier that indicated nearly zero percent sleep. The histogram looks like a noise-only recording.
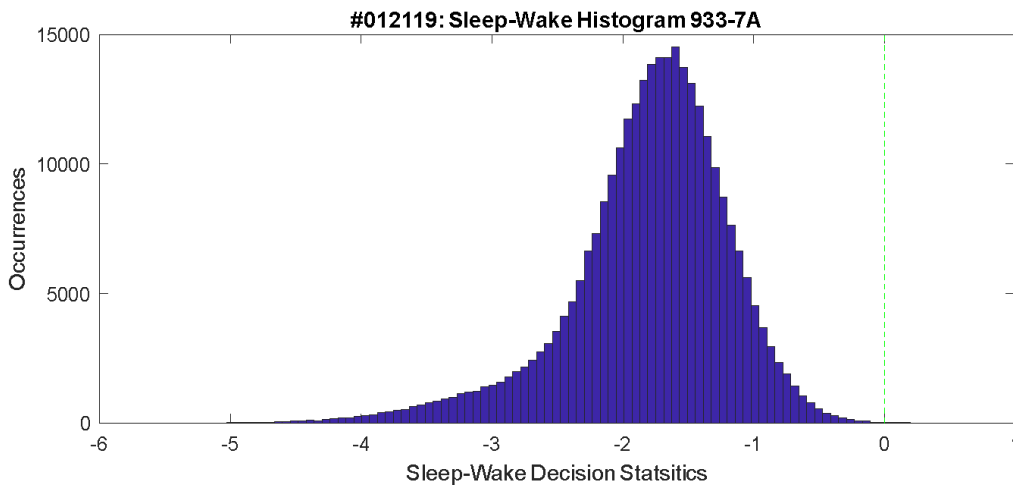
Fig A 8: An Example of a signal that appears to be noise-only.

Plotting the time series results in Fig A 9 below. It looks like there may be some strong wake signals near the light and dark phase transitions.

Fig A 9: Time series plot of the decision statistic which suggests strong wake signals.

Zooming in confirms this, *Fig A 10*.

Fig A 10: Zoomed in time series, which confirms strong wake signals near the transition.

Overlaying the pressure signal, typical wake and sleep transition patterns, based on signal envelope/amplitude characteristics, can be seen (*Fig A 11*).

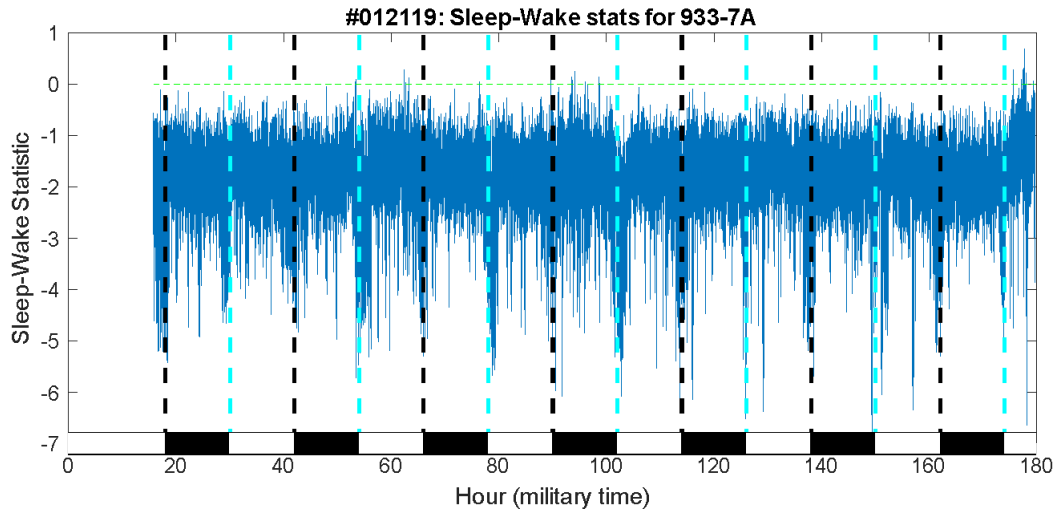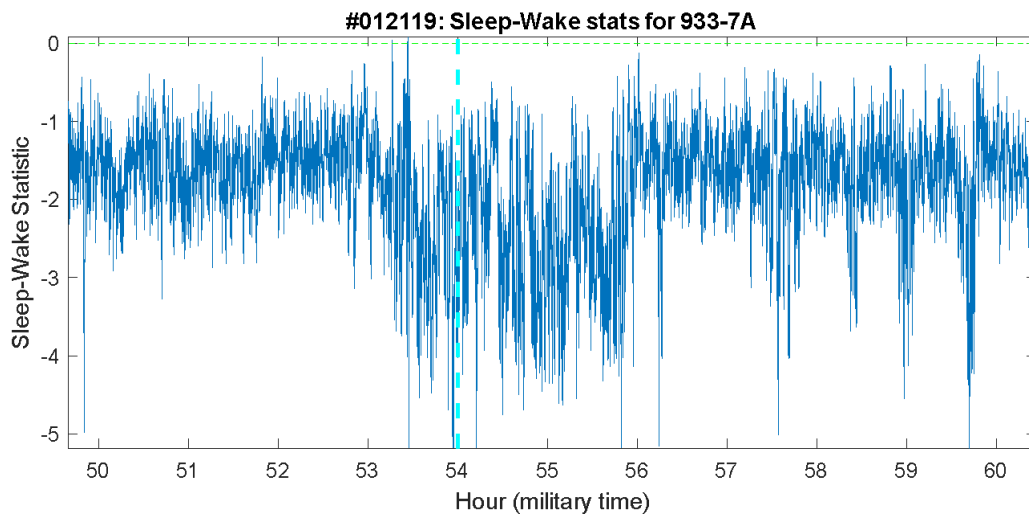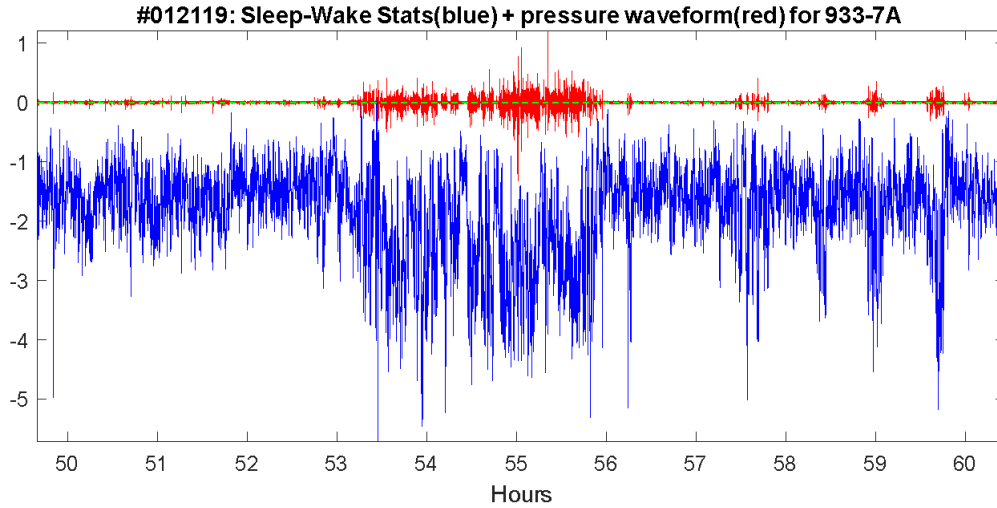Fig A 11: Pressure signal overlay for the plot in Fig A 10.

When zoomed further (*Fig A 12*), it appears that a threshold near -1.9 would separate most of the sleep and wake behaviors based on the envelope characteristics. This cannot be seen from the histogram, only in the time series data. When zoomed in, it can also be seen that the weak signal during sleep is corrupted by the noise floor in the recording. Therefore, a sleep decision was not strongly registered with a higher value decision statistic.
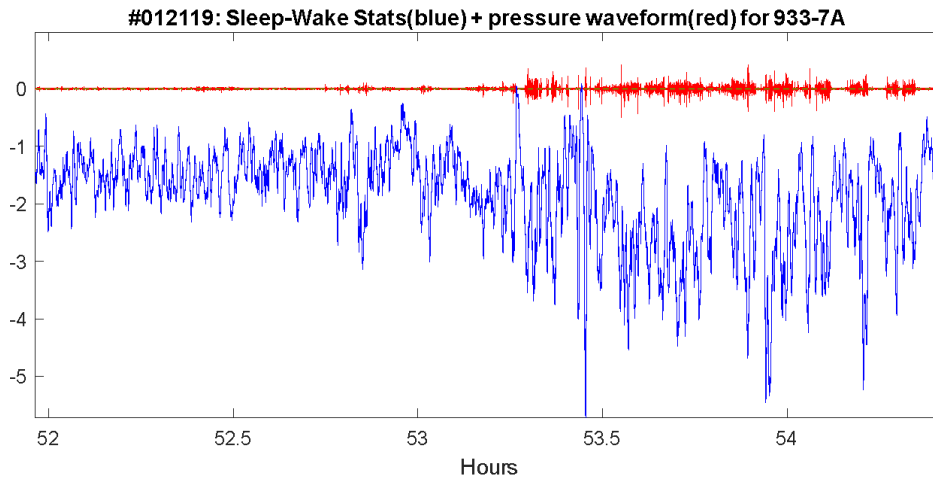
Fig A 12: Zoomed in figure to analyze where the decision threshold should be.

After changing the decision threshold, the figure will be updated, the threshold can be confirmed to have moved, and the plots can be inspected to see how it affected the results (*Fig A13*).
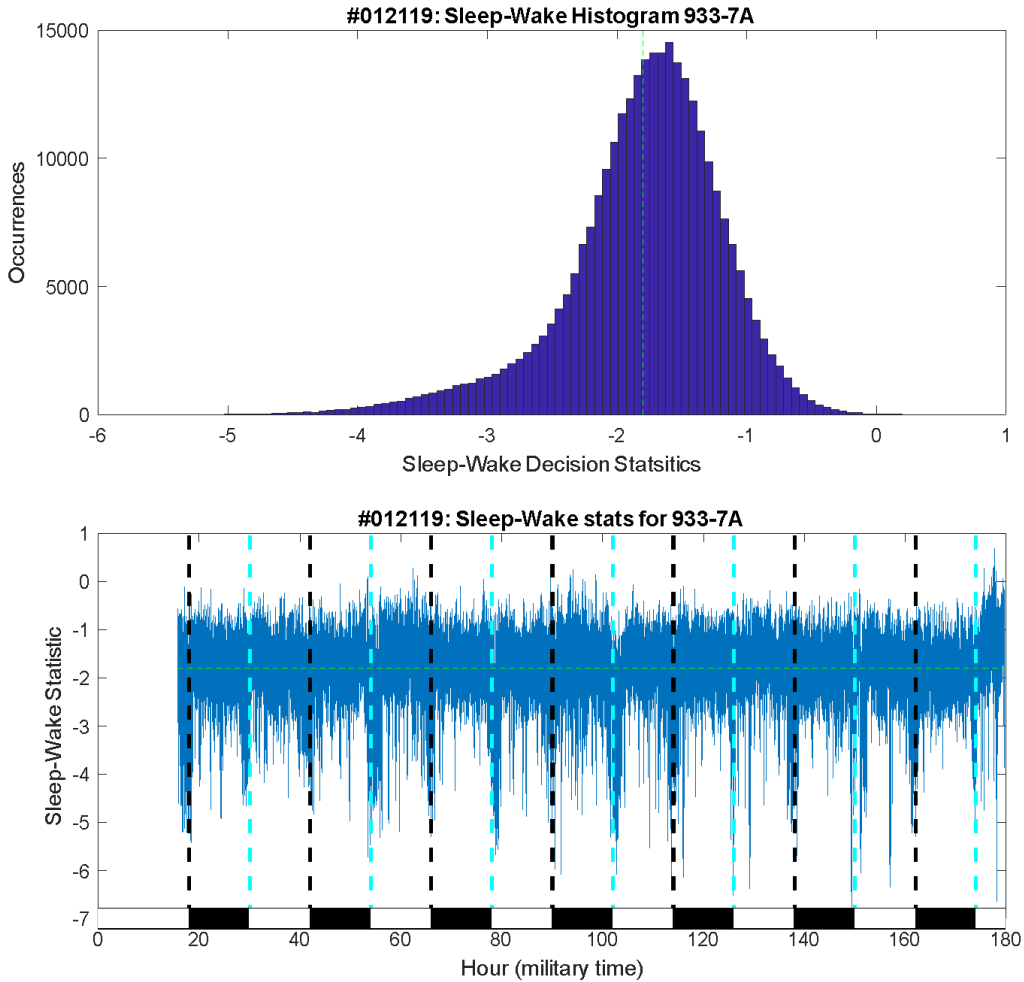
Fig A 13: Decision statistics in a histogram and time series to evaluate the new threshold.

Another example of an outline can be seen in *Fig A 14*. In this case, the sleep percentage was low, around 10%, and the histogram had an unusual multimodal appearance. This happened because the cage was subjected to regular machine noise during periods of light when the animal would typically be asleep. This created a lower mode in the sleep range. In this case, the noise disturbed the usual regularity of sleep and confused the automatic threshold setting. The histogram and time series in *Fig A 14* shows the original threshold location.
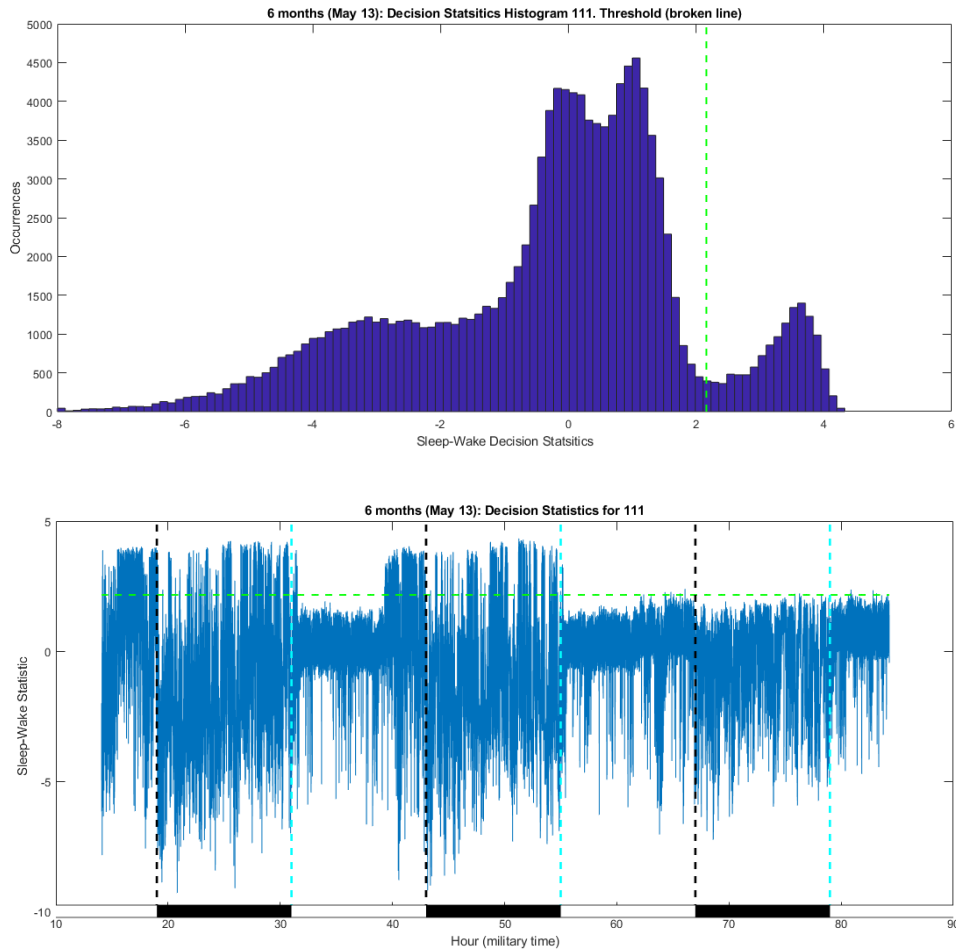
Fig A 14: Histogram and time series plot of a segment subjected to regular machine noise.

Observe, during the first two dark phases, there is a typical high-valued sleep decision statistics, here the noise was not influencing the decision. This is responsible for the smaller higher node, around four on the histogram. During the light phase, the periods of high and low decision statistics are not observable on this level. Zooming in on the time series and superimposing the sensor data (*Fig A 15*) better shows the regular machine noise during sleep bouts, in this case, the noise was three minutes on, then three minutes off, that is corrupting the sleep signal and making it look more like rest than sleep.
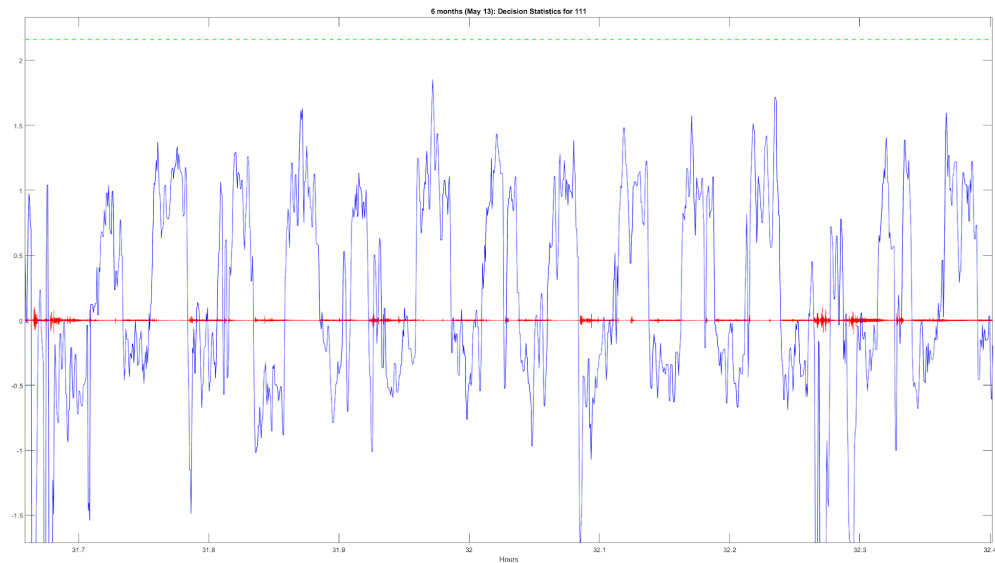
Fig A 15: Decision statistic zoomed in with pressure signal overlaid.

As can be seen, the threshold line was too high, improperly influenced by the disparity of sleep during the dark and light phases when noise was present. Also, the regular machine noise can be seen in the pressure signal, which causes the statistic to be driven lower, creating the middle two peaks in the histogram around zero and one. Here it would be recommended to move the threshold slightly below the statistical values driven down by the noise. If some short actual activity bouts look different and are higher than the machine noise, the threshold should exclude these from sleep. Moving the threshold too low would result in some of the wake, or periods of restlessness seen towards the end of *Fig A 15*, to be scored as sleep. In this case, a threshold of about -0.5 is recommended to try and catch all the sleep, but not overcompensate to include the borderline rest-sleep states as sleep. Below is the adjusted threshold on the histogram and time series in *Fig A 16.*
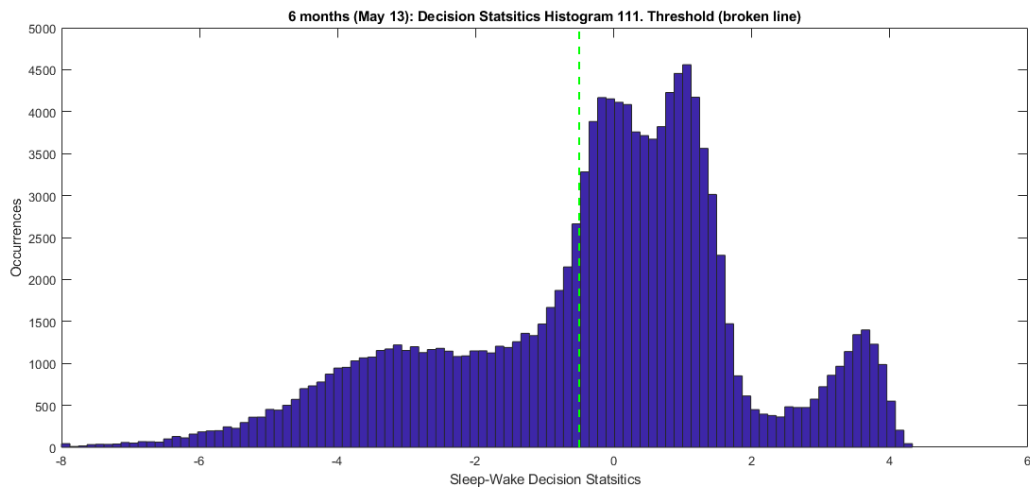
Fig A 16: Histogram and time series decision statistics after a threshold adjustment.

From the histogram, the two modes observed in the time series during the noise period are both to the right of the threshold. This threshold sensitivity can be tested as well. Some tweaking of it could be done, such as moving the threshold up a little closer to zero or down, to observe the changes in sleep percentages. The change in percentages will give a sense of performance ranges where the true values lie, ideally this range should be small for result repeatability.
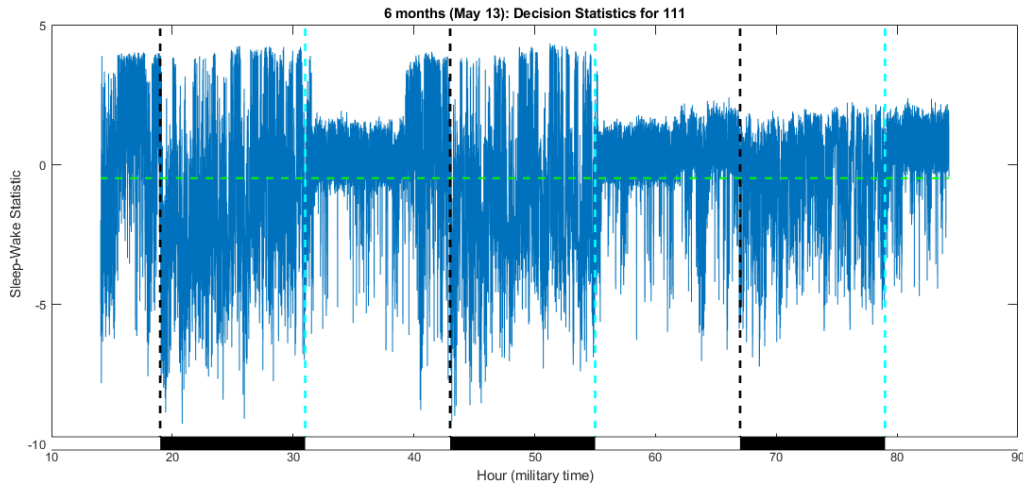
Appendix B – Performance Tests and Expected Accuracy

2-state (Sleep/Wake) algorithm for Mice

In 2018, a test was performed using 24 hours of electroencephalography (EEG), electromyogram (EMG), and piezo system data simultaneously, collected from 42 mice. All animal experiments were conducted at the University of Kentucky with prior approval from the university's Institutional Animal Care and Use Committee (IACUC). Two human sleep scorers used the EEG and EMG data to label the 24 hours of data as *Sleep* or *Wake* over 4-second intervals. The assessment was performed only on segments where both human scorers agreed (~95% of the data). The disagreement between the *PiezoSleep System* and human scoring was 7% for all sleep-labeled data and 7% for all wake-labeled. Since the error is balanced between the sleep and wake states, errors in statistics computed over greater time intervals will typically be less than this. For example, on a 24-hour period of computing percent sleep where sleep and wake states are equally likely, the errors in both directions offset each other and result in an expected error for daily sleep of about 1%. For a period in a dark phase where sleep is 30% likely and wake is 70% likely, the offset between the errors will be slightly biased, resulting in an error for a percent sleep estimation of about 3%. While these results can be expected for most

recordings, there are possibilities that noise and interference corrupt the pressure signals to the point of impacting the performance of the classification algorithm. In addition, the classification performance could potentially be impacted for a mouse with behaviors that are significantly different from the normal population, especially regarding their respiration patterns/rates. For this reason, the program provides multiple ways to look at the data and test for potential problems. These are described in this manual.

2-state (Sleep/Wake) algorithm for Rats

At the time of this writing, 2 published studies examine the agreement between the current PiezoSleep system (2.X8r and higher) and EEG/EMG scoring for rats. One study was published using 9 rats in 2022 (I. Topchiy *et. al.* 2022), and another used 14 rats for 7 days in 2021 (T. Vanneay *et. al.* 2021). This section summarizes these results and presents results from 8 rats performed internally by Signals Solutions.

The study by Topchiy *et. al.* used a corded system on 4 rats for 3 days, and a telemetry system on 5 rats for 16 days. Automatic sleep and wake scoring with EEG/EMG was performed using NeuroScore (3.0.7077 DSI) on 4-second intervals, and for 4 rats using a corded EEG/EMG system, 10-second intervals were scored by human assessment of the signals. In comparisons of results from the 3 systems, there was no significant difference in total sleep time or hourly sleep time. The was, however, a trend for NeuroScore results to score more sleep than PiezoSleep, and the human scored EEG/EMG results. In terms of accuracy in comparing 4-second intervals there was an agreement between sleep and wake scores of 85.6% for the corded system and 80.8% for the telemetry system.

The study by Vanneay *et. al.* tested the PiezoSleep system with 14 rats for 7 days using 10-second scoring intervals. Two light-dark cycles were used, where 7 rats were tested with LD12:12 and the other 7 rats with LD16:8. EEG/EMG sleep-wake was scored by human assessment of the signals over 10-second intervals. For both light cycles and both methods, there was no significant difference in total sleep time or hourly sleep percentages. The accuracy for sleep-wake scoring on 10-second intervals was 81.9% for LD12:12 and 84.9% for LD16:8.

In 2019, the *PiezoSleep* system was tested at Signal Solution for 8 rats using 24-hour recordings of electroencephalography (EEG) and electromyogram (EMG), simultaneous with the *PiezoSleep* signals. Data were collected and EEG/EMG signals were scored automatically on 4-second intervals with NeuroScore at the Center for Sleep and Health Research, located at the University of Illinois in Chicago. In this evaluation, adjacent scores from PiezoSleep, which are given every 2 seconds, were combined so they could be compared to 4-second EEG/EMG scored intervals. Intervals that were split were not used in the evaluation since the NeuroScore results did not match this resolution. In addition, potential misalignments between the scored time series were compensated for by performing a correlation between the NeuroScore and 4-Second interval PiezoSleep results. The misalignments could result from the clocks used for the EEG/EMG and the piezo system being off slightly and filtering/buffering delay differences in the

outputs of the 2 systems. The shift corresponding to the maximum correlation was applied to align the EEG/EMG scores with the PiezoScores. The adjustment was typically no more than 3 sample points. This resulted in a slightly higher score than the studies mentioned above. The accuracies for the 8 rats ranged from 85% to 91% with an average of 89%. The percent error was 8.3% during sleep and 14.7% during wake. The total sleep time was 59.4% for the EEG/EMG scoring and 60.4% for the PiezoSleep System scoring.

While these results can be expected for most recordings, noise and interference could corrupt the pressure signals to the point of impacting the performance of the classification algorithm. In addition, there can potentially be rodents with behaviors that are significantly different (especially respiration patterns/rates) than the normal population. For this reason, the program provides multiple ways to look at the data and check for potential problems. These are described in this manual.

3-state (Wake/NREM/REM) algorithm for mice

The data set used to validate the 2-state performance was used to develop an ensemble decision tree algorithm for classifying wake, NREM, and REM vigilance states. After developing a decision tree classifier based on piezo signal features, the system was sent to an independent laboratory for validation (Paul Franken, Centre Intégratif de Génomique, Bâtiment Le Génopode, Université de Lausanne, Lausanne, Suisse). At these laboratories, simultaneous EEG/EMG and piezo system recordings were made for 4 mice over a 24-hour period. The human-scored EEG/EMG at 4-second intervals were up-sampled to match the decision tree algorithm classification at 2-second intervals. The resulting performance based on matching 2-second intervals was consistent with the training and testing in the development stage. Since wake was detected from the 2-state algorithm, the performance for wake states is the same as it was in the 2-state. For the sleep detections, the decision tree algorithm was applied to classify REM and NREM detections. For all human-scored NREM intervals (based on EEG/EMG scoring) there was a 12% disagreement with the automatic piezo-based algorithm scoring, and for REM, there was a 52% disagreement. The low agreement for the REM numbers suggests that the patterns picked up from pressure on the cage-floor sensor (from breathing and weight shifting) do not have as strong a correlation to the vigilance states as do the sleep and wake behaviors. In addition, since REM states typically occur only about 6%, it does not take many errors to increase the disagreement rate relative to wake and NREM.

The REM patterns typically detected by the piezo-based algorithm are respiration patterns that become more irregular during detected sleep. Cage-floor pressure changes from the thorax motion (due to respiration) during REM exhibit rate changes over short-time intervals and low-level amplitude variations more so than NREM. This pattern does not have a strong one-to-one correlation to the human-scored REM intervals from the EEG/EMG signals; however, piezo-based detections typically increase around and during REM events. Figure B 1 shows an example of the pressure waveform overlaid with the human and piezo system scoring states. Observed in the blocks of human-scored REM states, is an increase in the piezo system

detections; however, it is not contiguous over this block, as the patterns of irregularity are not maintained over all short intervals in this block. In addition, false positives typically occur around transitions from wake to sleep and sleep to wake, as this is usually accompanied by more irregular breathing. This accounts for most of the disagreements between the human and piezo-based systems. To see the impact of disagreements at transition points, the disagreement rate computation at transitions is modified to include a consistent REM detection if a human-scored REM occurrence is within 1 detection interval (2 seconds) of the piezo system detection. With this rule, the disagreement drops to 38% overall.
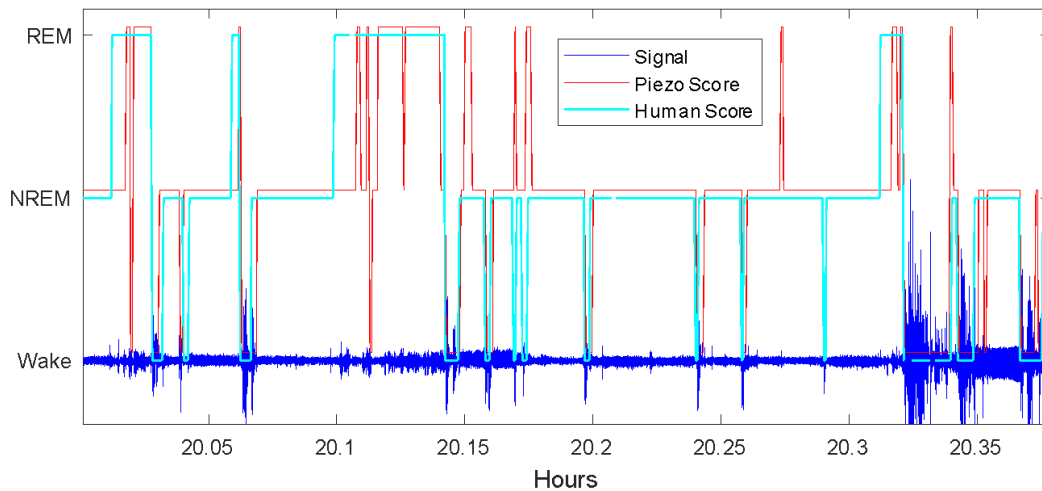
Fig B 1: Piezo system pressure signal overlaid with human EEG/EMG scoring and piezo system scoring.

To see how the algorithm can track trends in REM behavior over longer periods, the percent REM was computed over 1-hour intervals, and overlapping intervals were incremented every 12 minutes to create the percent REM plots over the 24-hour period as shown in Fig. B 2. This was performed on the human and piezo systems, scored results, and plotted for comparison. Figure 2a was the best case of the validation set. There is a slight overprediction on the percent REM for most of the intervals, but the percentages were within a few percent over most of the scoring period. 2b was the worst case of the validation set. There is almost a 10% difference on

some for early REM increases (peaks). In both cases, however, the increases and decreases in percent REM over time show good agreement.
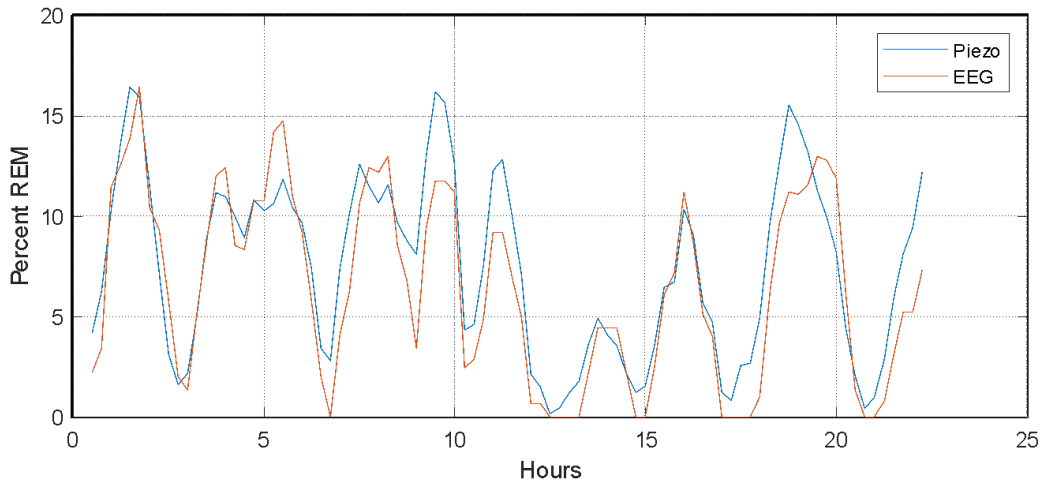
(a)
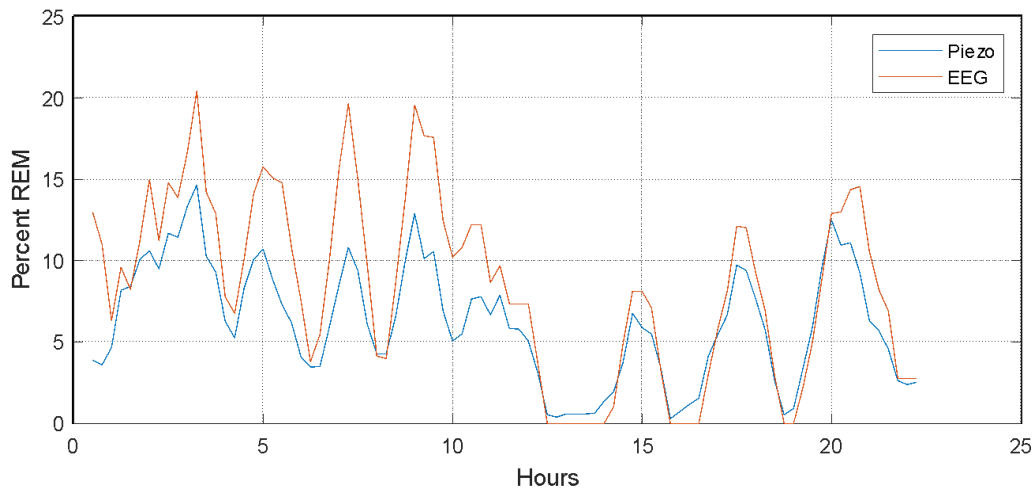
(b)

Fig B 2: Comparison of percent REM sleep over 1-hour intervals for human-scored EEG/EMG and automatic piezo system scored. (a) Best match in the validation set (b) worst match in the validations set.

In conclusion, the REM detection feature has value in detecting changes in patterns of REM-like behavior (irregularity in breathing during sleep) over time; however, it is limited in comparing quantitative amounts of REM sleep from animal to animal. These results were obtained using Signal Solution's custom cages with the film sensors. Since subtle changes in the breathing signal is an important factor in the automatic 3-state algorithm, environments, and/or cage systems with noise levels on the order of the mouse breathing signal can significantly impact

the algorithm performance. If you are unsure of the noise levels on your system, we recommend contacting Signals Solutions to analyze a recording.

Appendix C – Feature Breakdown

Features	SleepStats 2.X8	SleepStats 4 LITE	SleepStats 4 BASIC	SleepStats 4 PRO
PLOTS				
Sleep/Wake Plots				
Percent Daily Sleep	Yes	Yes	Yes	Yes
Percent Daily Sleep in Light and Dark	Yes	Yes	Yes	Yes
Sleep/Wake Decision Statistic Plot	Yes	Yes	Yes	Yes
Sensor Data Overlay	Limited	No	Yes	Yes
Breath Rate Overlay	Yes	No	Yes	Yes
Activity Overlay	No	No	No	Yes
Environmental Sensor Overlay	No	Yes	Yes	Yes
Percent Sleep/Wake Over Sliding Interval	Wake Only	Yes	Yes	Yes
Variable Window and Increment	In Options Menu	Yes	Automatic Graphic Update	Automatic Graphic Update
Bout Length Histograms	Yes	No	Yes	Yes
Time Period Switch (Separate L/D)	Before Figure is Generated	No	Automatic Graphic Update	Automatic Graphic Update
Sleep or Wake Option	Before Figure is Generated	No	Automatic Graphic Update	Automatic Graphic Update
Normalization Method Option	Before Figure is Generated	No	Automatic Graphic Update	Automatic Graphic Update
Bout Computation Method	No	No	Automatic Graphic Update	Automatic Graphic Update
3-State Stat Plots (For Mice Only)	No	No	No	Yes
Percent Daily 3-State	No	No	No	Yes
Percent Daily 3-State in Light and Dark	No	No	No	Yes
3-State Decision Statistic Plot (For Mice Only)	No	No	No	Yes
Sensor Data Overlay	No	No	No	Yes
Percent 3-State Over Sliding Interval (For Mice Only)	No	No	No	Yes
Variable Window and Increment	No	No	No	Automatic Graphic Update
Bout Length Histograms (For Mice Only)	No	No	No	Yes
Time Period Switch (Separate L/D)	No	No	No	Automatic Graphic Update
Sleep or Wake Option	No	No	No	Automatic Graphic Update
Normalization Method Option	No	No	No	Automatic Graphic Update
Bout Computation Method	No	No	No	Automatic Graphic Update
ACTOGRAM	No	No	No	Yes
EXPORTS				
2-State Exports				
Sleep-Wake Decision Statistic	No	No	Yes	Yes
Sleep-Wake Decision Histogram	Yes	No	Yes	Yes
Percent Sleep over User Defined Interval	Yes	Yes	Yes	Yes
Percent Sleep-Wake Segmented by Light & Dark	No	No	Yes	Yes
Time Stamped Sleep Bouts	No	No	Yes	Yes
Segmented Bout Stats	Yes	No	Yes	Yes
Percent Sleep/Wake Over Sliding Interval	Wake Only	No	Yes	Yes
Sleep-Wake Bout Histogram Segmented by Light & Dark	No	No	Yes	Yes
Breath Rate Exports				
Breath Frequency	No	No	Yes	Yes
Breath-Rate Stats	No	No	Yes	Yes
Activity Exports				
ClockLabs formatted Export	No	No	No	Yes
Segmented Activity Stats	No	No	No	Yes
Environmental Sensor Exports	No	No	Yes	Yes
Environmental Sensor Data	No	Yes	Yes	Yes
Enviromental Sensor Stats	No	Yes	Yes	Yes
3-State Exports				
Time Stamped 3-state Bouts	No	No	No	Yes
Percent 3-State per User Interval	No	No	No	Yes
Percent 3-State Segmented on Light & Dark	No	No	No	Yes
Processing Options				
GUI for Quick Changes and Automatic Graphic Update Plot Updates	No	Yes	Yes	Yes
Daily Cycle Duration (can set different from 24 hours)	No	No	Yes	Yes
Light/Dark Determined by Light Sensor on Environmental Sensor	No	No	No	Yes

References

(I. Topchiy *et. al.* 2022) Topchiy I, Fink AM, Maki KA, Calik MW. Validation of PiezoSleep Scoring Against EEG/EMG Sleep Scoring in Rats. Nat Sci Sleep. 2022 Oct 20;14:1877-1886. doi: 10.2147/NSS.S381367. PMID: 36300015; PMCID: PMC9590343.

((T. Vanneay *et. al.* 2021) Vanneau T, Quiquempoix M, Trignol A, et al. Determination of the sleep-wake pattern and feasibility of NREM/REM discrimination using the non-invasive piezoelectric system in rats. J Sleep Res. 2021;30(6):e13373. doi:10.1111/jsr.13373.