



SIGNALSOLUTIONS
Sensor Systems, Software, Sleep

SleepStats
Signal Solutions, LLC
User Manual

for SleepStats Version 2.x8

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Sleep Stats Data Explorer

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1 Introduction:

The **SleepStats** data explorer software features programs that open and process multi-channel files created by the high-throughput **PiezoSleep** data recording and real-time monitoring system. The functions in this software primarily:

- plot graphical descriptions of sleep and wake behavior.
- create CSV files with statistics characterizing sleep and wake behaviors.
- plot classifier data and sensor signals to check on signal quality.

The functions are accessed through a menu driven program called *SleepStats*.

Performance Tests and Expected Accuracy:

In 2018 a test was performed using 24 hours of electroencephalography (EEG), electromyogram (EMG), and piezo 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. 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, error on statistics computed over greater time intervals will typically be less than this. For example, on a 24 period of computing percent sleep where sleep and wake states are equally likely, the errors in both directions will offset each other resulting in an expected error for daily sleep of about 1%. For a period of time 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 could corrupt the pressure signals to the point of impacting the performance of the classification algorithm. In addition, there can potentially be a mouse 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 test for potential problems. These are described in this manual.

Installation:

Download the sleepstatsinstaller from the URL provided with your purchase and archive to a directory of your choice. Execute the downloaded file. The computer may warn you about the software, depending on your security settings and operating system. If this is your first program installation, it will download files from the Mathworks web site (so you must be connected to the internet for the first installation). The transfer requires about a 500 MB download. In the rare event that Mathwork's server is slow or the internet traffic is congested, the download may time out and give an error. In that case, the servers may be too busy. If you wait a few hours and try again it usually clears up. Once fully downloaded, the installation script will continue with a prompt to suggest a folder to save the executable in. You can change this if necessary. For easy access to the program, select the *Add shortcut to Desktop* box. If you don't select this, the program

will be available through the *All apps* option on your desktop start menu. The installation script will then indicate that the program requires MATLAB runtime. Select the install option and the program will download necessary 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.

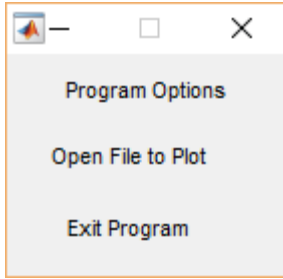


Figure 1. Initial menu to quit or get file.

2 SLEEPSTATS Functions

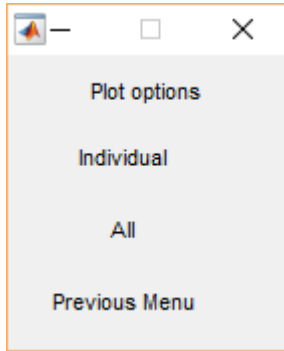
This program is used for checking the integrity of data, observing sleep-wake parameters, generating statistics, and creating CSV files for further analysis in spreadsheet and database programs. It can be executed by clicking on the *sleepstats* icon on the desktop or accessed through the app menu. The program initially generates the menu shown in Fig. 1. Click the text *Open File to Plot* to select a feature vector file. By default, all files listed in the navigator window have a *FeatVecfb* extension. This file contains the feature vectors created by *PiezoSleep*, which are used to compute decision statistics and thresholds for determining the sleep or wake condition for each individual 2 second intervals. It may take a few minutes to open the file depending on size and computer speed.



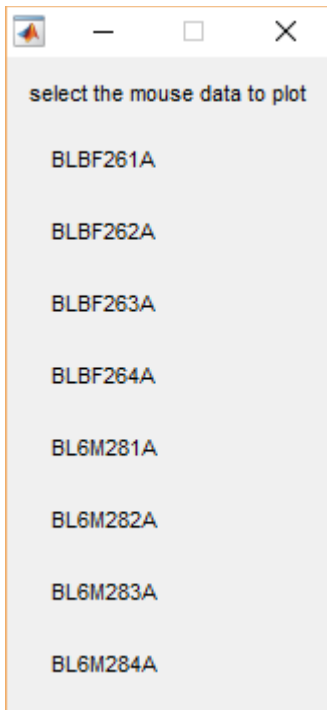
Figure 2. Options menu after a *FeatVecfb* file has been successfully opened.

Menu Overview: Once the file has been successfully opened, the menu shown in Fig. 2 will appear. The first 2 options present plots of the sleep-wake decision statistics, thresholds, breath rate, and the sensor data. These are useful for verifying the integrity of the data. Options 3 through 7 create plots of sleep-wake characteristics, such as percent sleep and wake over a specified interval, and bout lengths. Options 8 through 11 export statistics to CSV text files for importing into spreadsheet or database programs. The next 2 options provide figure operations. The program opens a new figure window with each graphic request, and they remain on the desktop until closed. Figures can be deleted individually by clicking the X in the upper right corner of the figure window, or all figures can be closed by clicking the *Close All Figures* button. If you click on the *Copy Selected Figure* button, then either the last figure created or the last figure selected will be copied to the clipboard for pasting into another program. The menu item “file” on the top figure bar provides options to save the figure in various formats through the save or save as options. The *Change Processing Options* button presents a submenu with options to change parameters that

control the plotting and processing of the data, such as decision thresholds and bin intervals. The *Quit/Get New File* button returns the previous menu (Fig. 1) either to exit the program or open a new file.



(a)



(b)

Figure 3. Channel selection menu. (a) individual or all channel options (b) individual channel selection prompt with default mouse labels.

2.1 *Plot Sleep-Wake Decision Statistics*

The *Plot Sleep-Wake Decision Statistics* provides options to plot decision statistics computed from the feature vectors. Users can view these plots to determine if the threshold should be changed or if something unusual happened to the data (deceased mouse, bad sensor, noise/interference, etc.). This option creates the submenus shown in Fig. 3.

The user can select an individual channel for plotting, or automatically generate all plots (one figure window corresponding to each animal). The figures are labeled as the animal IDs given in the *PiezoSleep* data acquisition program. If special labels were not created at the beginning of the *PiezoSleep* data recording session, default labels are used (*Animal1*, *Animal2*, *Animal3* ... up to the total number of channels). If the *Individual* option is selected from the menu in Fig. 3a, the prompt in Fig. 3b will appear with a list of channel labels.

Figure 4 shows the resulting plots with a linear time axis (x-axis) in hours, starting at the hour of the day (in military time) when the recording began. The y-axis is the decision statistic from the sleep-wake classifier. The larger the value, the more likely that sleep occurred at that time. The decision threshold is computed adaptively to account for variations in mouse behavior and weight based on the clustering of decision statistics. The threshold is shown as the broken green horizontal line. Decision statistics above this line are classified as sleep. The thresholds for individual channels can be changed from the *Change Processing Options* menu button shown in Fig. 2. The sleep-wake classifier was designed to generate good performance with decision thresholds near 0. The adaptation offsets it slightly based on the clustering of the decision statistics for recordings over 24 hours. For recordings less than 24 hours, a default threshold of 0 is used. For reference to the light/dark sequence applied during the recording, dark onset and light onset times are marked with broken black and cyan vertical lines, respectively.

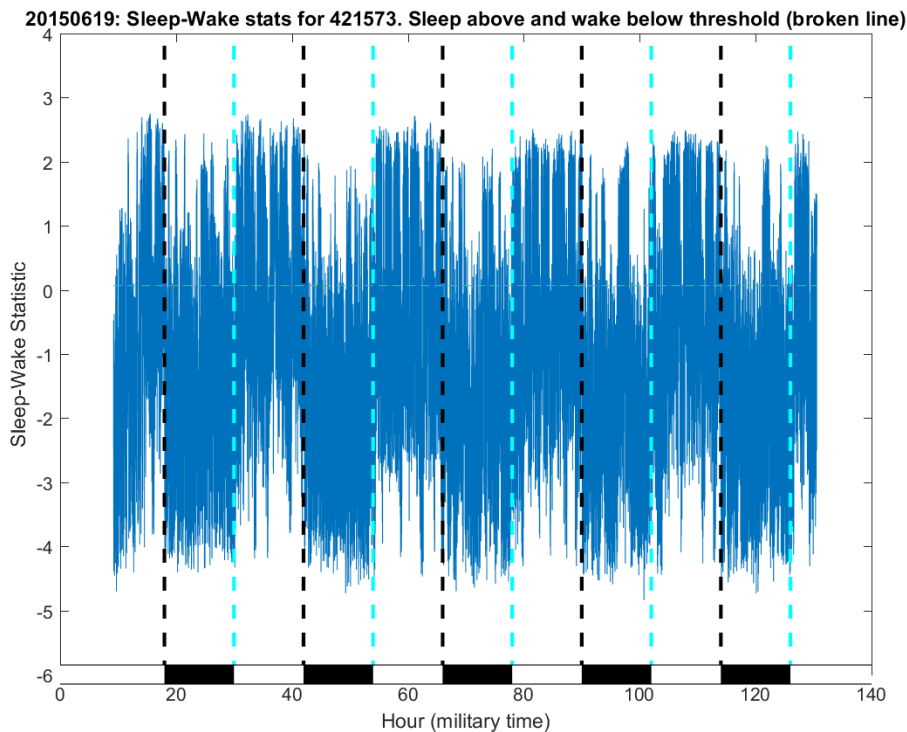


Figure 4. Sleep-wake statistics plotted over the full duration of the experiment. Black vertical lines mark the light-to-dark transitions and cyan vertical lines mark the dark-to-light transitions. The light green horizontal broken line is the decision threshold.

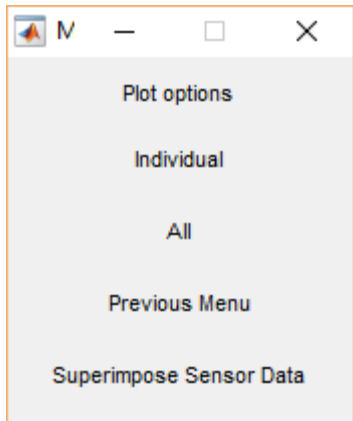
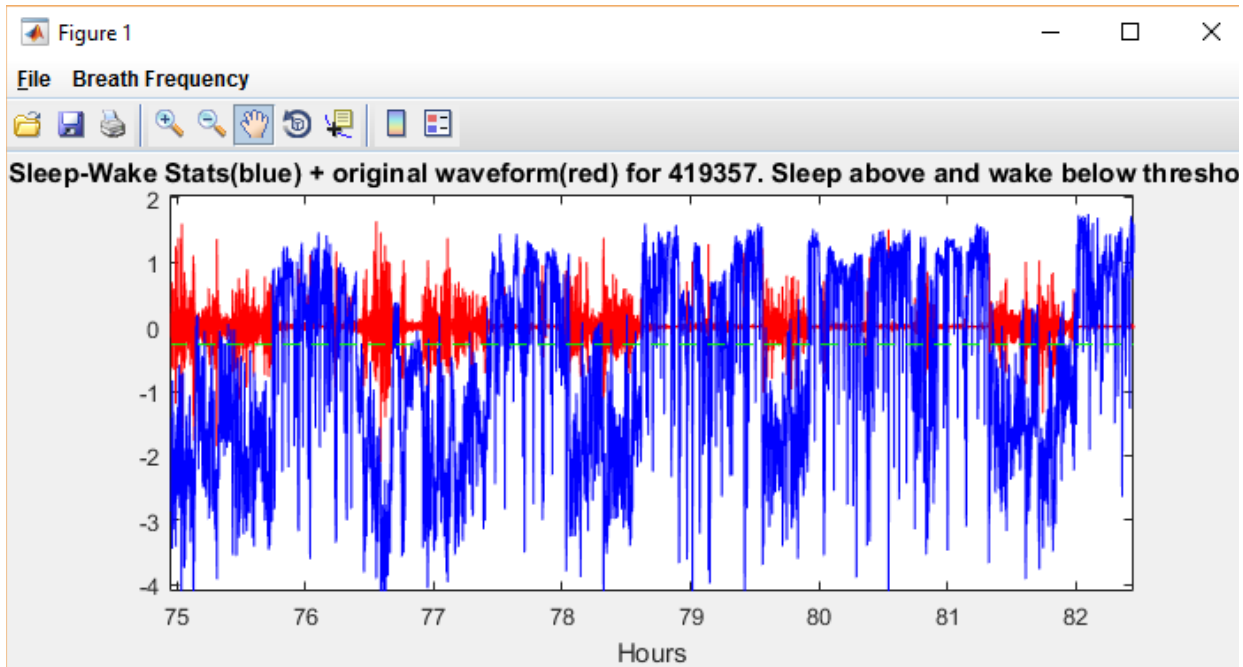
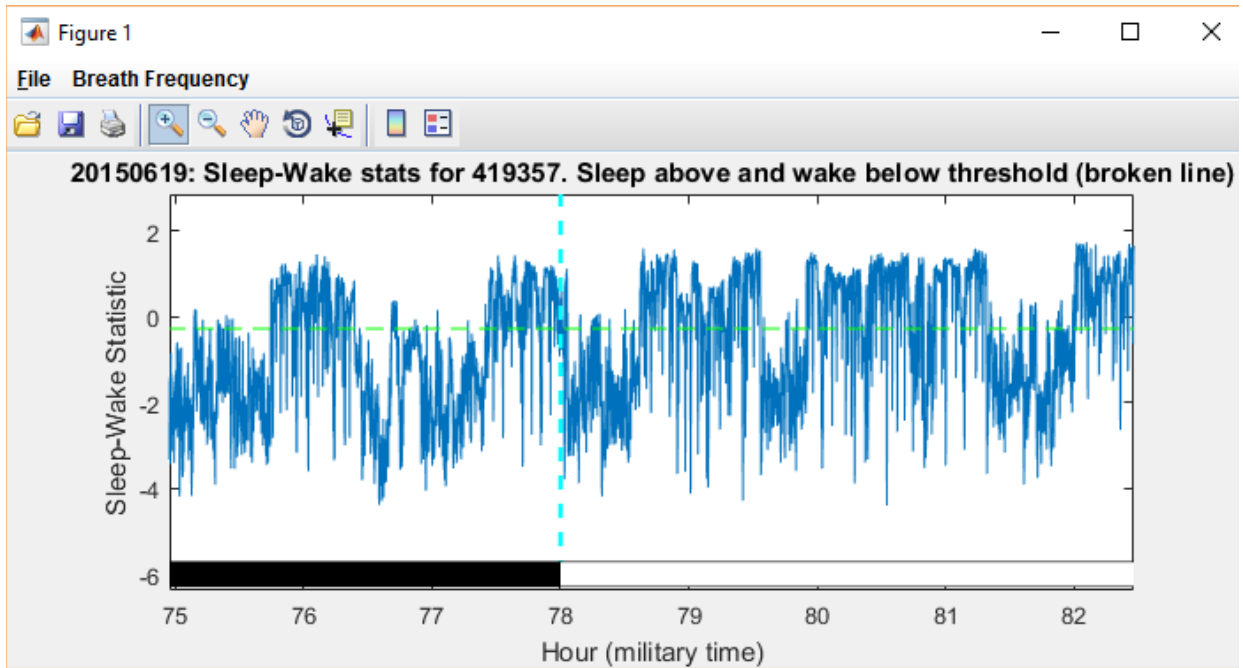
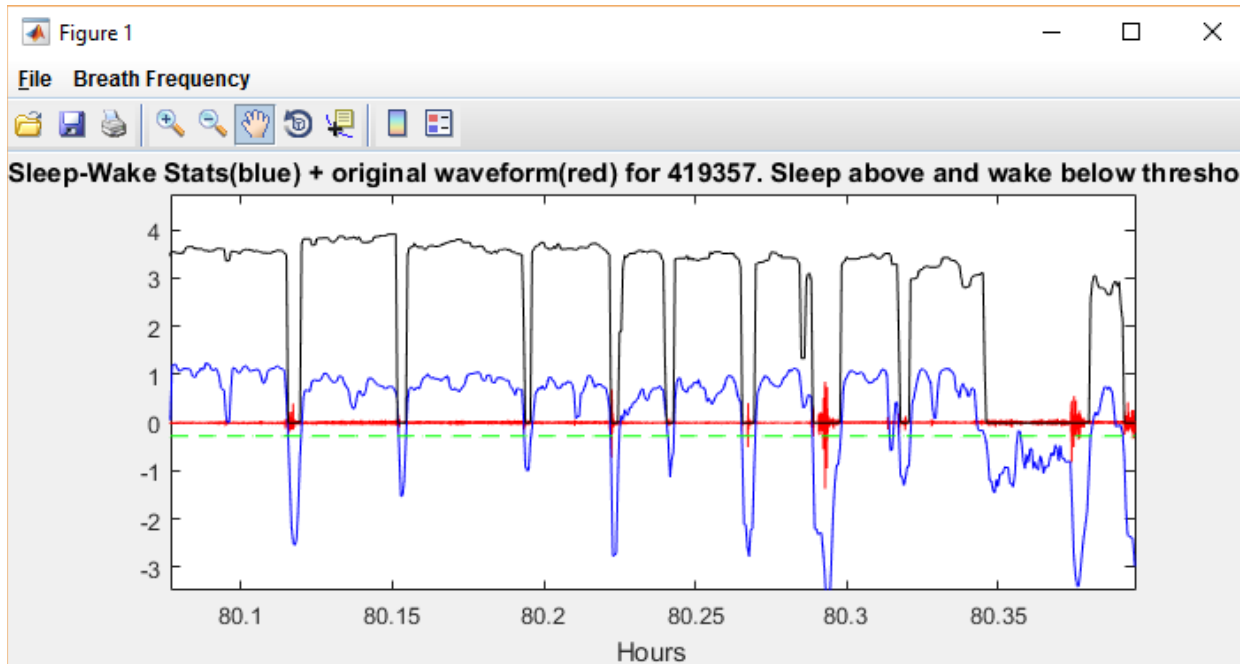


Figure 5. Channel selection submenu of Fig. 3a when the individual plot is open. Option to superimpose data from the piezoelectric sensor.

After plotting an individual channel, the submenu shown in Fig. 5 appears to provide an option for superimposing the sensor data over the decision statistics. To use this option, the pressure signal data file must be present on the local computer. If the base name of the opened *FeatVecfb* file does not match the base name of the sensor signal file in the same directory (with a *binfb* extension), then a file navigator window will appear so the sensor signal file can be selected by the user. If the file is not present, the file navigator window can be closed to return to the menu in Fig. 5. The *PiezoSleep System* automatically creates these files with common base names during the data capture process.

When using the *Superimpose Sensor Data* option, it is best to zoom in on a smaller portion of data (~4 hours or less) before clicking on the *Superimpose Sensor Data* button. If the time axis shown in the figure is more than 6 hours, it may take a while to load, and limit the details visible in the plot. To zoom in on a portion of the plot, click on the magnifying glass icon with the + sign in the figure toolbar, then click and hold down the left mouse button and drag the pointer over the area of interest. Release the mouse button and the figure will expand the area defined by the mouse drag. (To get the full axis back, right click the mouse on the figure and select the *Restore to Original View* option.) Once the region of interest fills the figure, click the *Superimpose Sensor Data* button to generate the overlay of the pressure signal from the sensor on the decision statistics as shown in Fig. 6b. Breath rates are computed in *PiezoSleep* and saved in a file with a *Feat* extension. If this file is present, the *Breath Frequency* menu item will appear on the top figure menu bar. If selected, the breath rate will be superimposed on the plot, as shown in Fig. 6c.



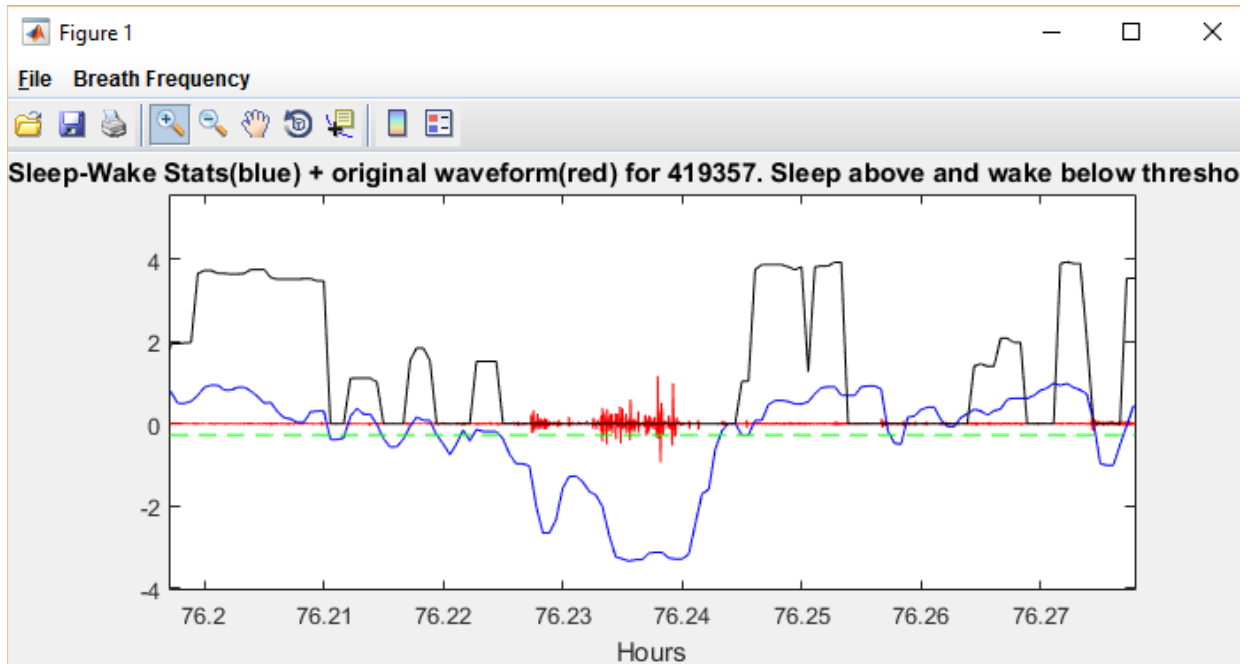


(c)

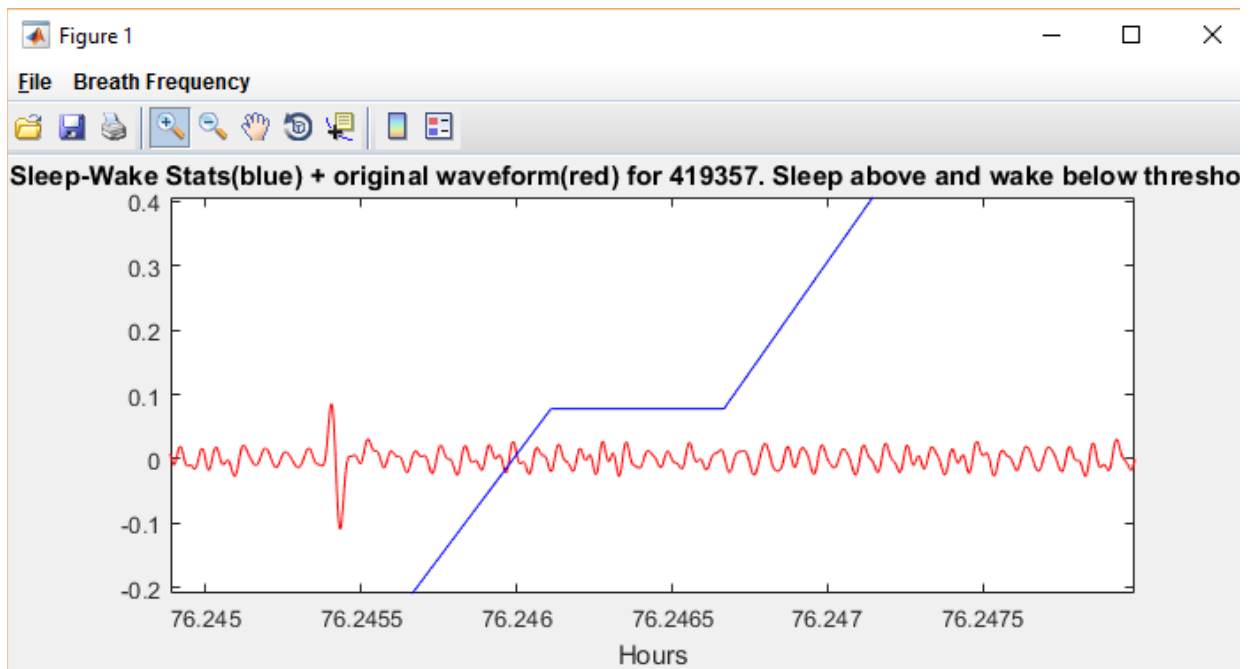
Figure 6. (a) Zoomed in plot of decision statistics (before selecting the *Superimpose Sensor Data* button). (b) Same figure with original piezoelectric sensor data superimposed in red. (c) Breath rate superimposed in black over decisions statistics.

If the time axis you selected is greater than 5 hours, it will warn you that you may not get the best plot or it may take a while to generate. This is only a warning. If you still want to see the data overlaid, just click “Continue”. If you click “Quit”, you can return to the figure to zoom in on the region of interest and request the overlay again.

Figure 7 shows examples of zooming in on smaller regions of the plot shown in Fig. 6. The result of zooming in on an interval of mostly sleep for the larger time scale is shown in Fig. 7b. Decisions are made every 2 seconds, so the discontinuities in the blue line denote 2 second intervals. Note the periodic rhythm of the breathing motion (~ 3 Hz) with amplitude regularity. This is a typical sleep signature.



(a)



(b)

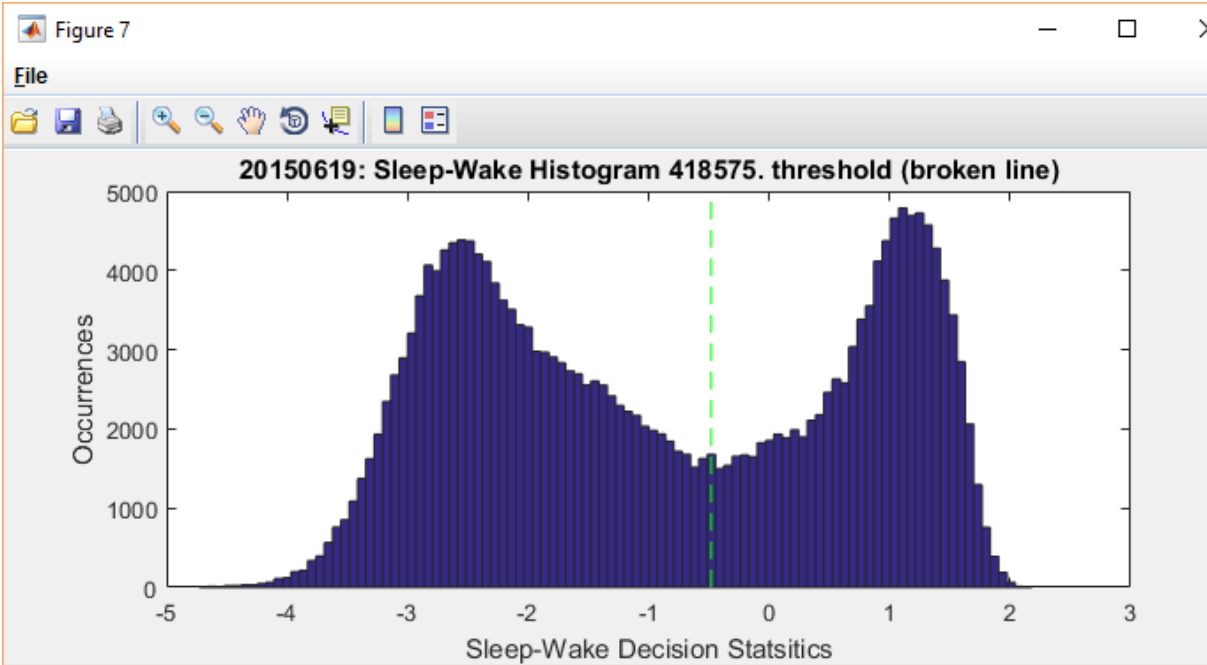
Figure 7. (a) Zoomed in segment on a region corresponding to wake near the center, showing the original piezoelectric signal (red) with the corresponding decision statistic (blue). (b) Zoomed in segment on a region corresponding to mostly sleep.

Larger amplitude variations and frequency changes correspond to more random movements that are consistent with an awake mouse. For a segment of time in the middle of Fig. 7a, the pressure signal does not have a characteristic sleep pattern. As a result, the decision statistics are less than zero, indicating that the mouse was awake.

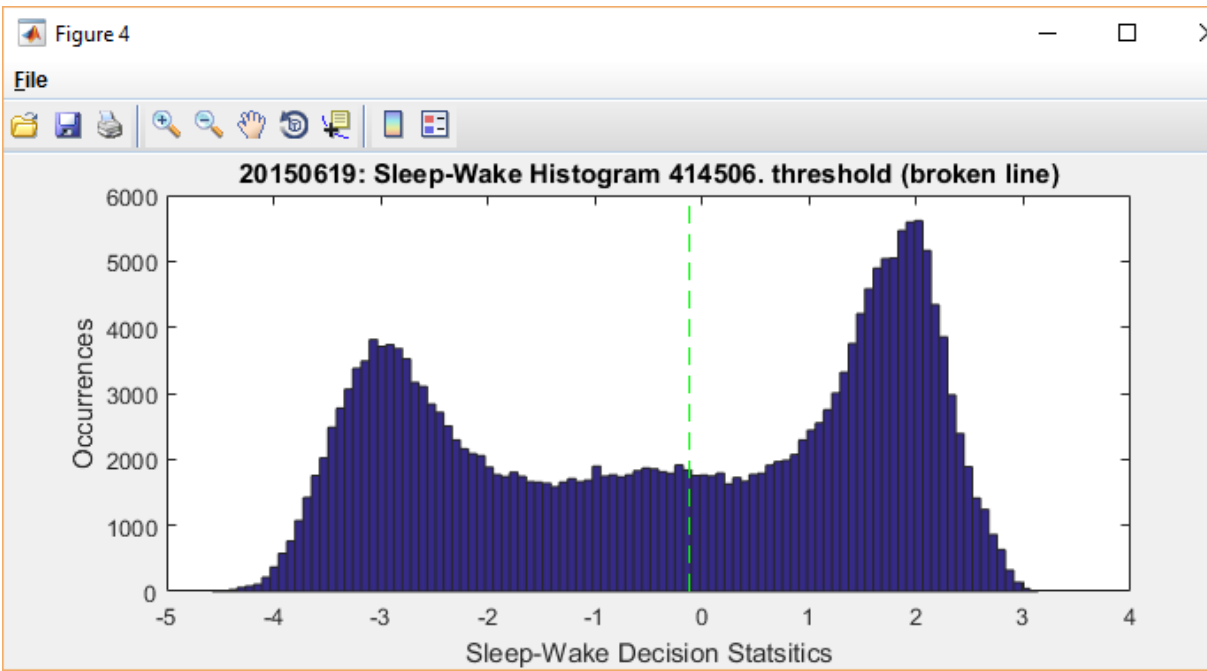
2.2 *Plot Sleep-Wake Decision Histogram*

The *Plot Sleep-Wake Decision Histogram* button in the options menu (Fig. 2) generates graphs that are useful for assessing how well the classifier distinguishes between sleep and wake behaviors. For a mouse with normal sleep and wake patterns, decision statistics over the course of 24 hours should accumulate around low values for wake and high values for sleep. The result is a bimodal histogram of the decision statistics. Figure 8 shows examples of a good separation of the bimodal peaks for decision statistics. The adaptive threshold is also shown located at the minimum or saddle point, which should minimize error. For typical mice recorded over a 24 period or greater, a strong clustering to either side of 0 suggests reliable results. However, for more varied behavior during the wake state activity, the histogram may not show a sharp peak for wake or may even have multiple modes during wake. This can sometimes cause a threshold setting to be less than optimal. In this case it is a good idea to check the time series (as in Figures 6 and 7) plots to make sure the sleep-like and wake-like waveforms are being separated properly. See Appendix A1 for information on how to manually set thresholds.

Examples of histograms that are not consistent with reliable recordings of sleep and wake activity are shown in Fig. 9. These were recorded with no mice in the cage, and therefore are dominated by noise and ambient motion/vibration in the room. Figure 9a shows a typical response to noise. Note the unimodal Gaussian-like form of the histogram in the negative value range. This is consistent with a random response to the noise floor. While the noise signal amplitudes are low, like the sleep signals, they do not have the consistency in amplitude and frequency of sleep signals, and therefore are classified as wake. The histogram in Fig. 9b is also noise, but may have picked up some low-level periodic noise, possibly from an air vent or ambient vibrations that created a weak repeatable pattern. The fact that the decision statistics are not positive in this case, suggests that this interference will not impact the accuracy of the sleep wake system. A recommended test after setting up the *PiezoSleep* system, is to let it run for a day or 2 without mice and look at the histograms. The channels should generate histograms that tend to be unimodal and dominated by negative values. If a significant fraction of decision statistics is positive when no mice are in the cage, or if the histogram has an odd shape, the cage placement and electronic connections should be checked. Make sure amplifier cables are seated well into their sockets. Also check to make sure there are no vibrations from machines or shelf rocking/vibrating from air currents or other motion in the room.

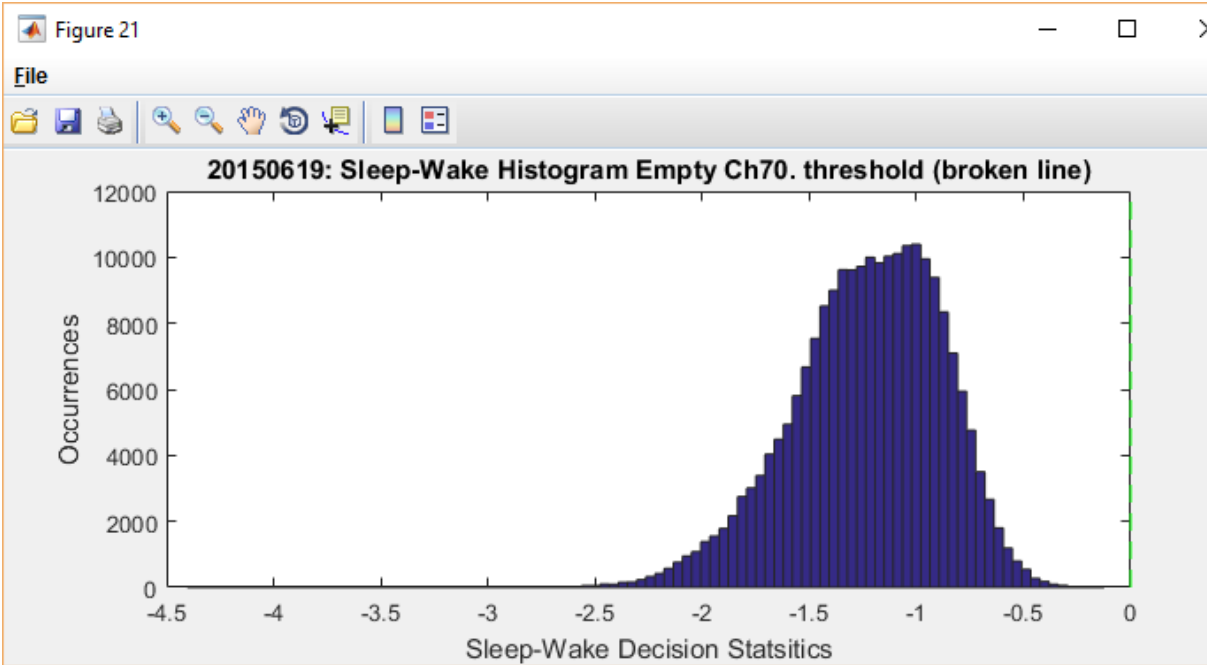


(a)

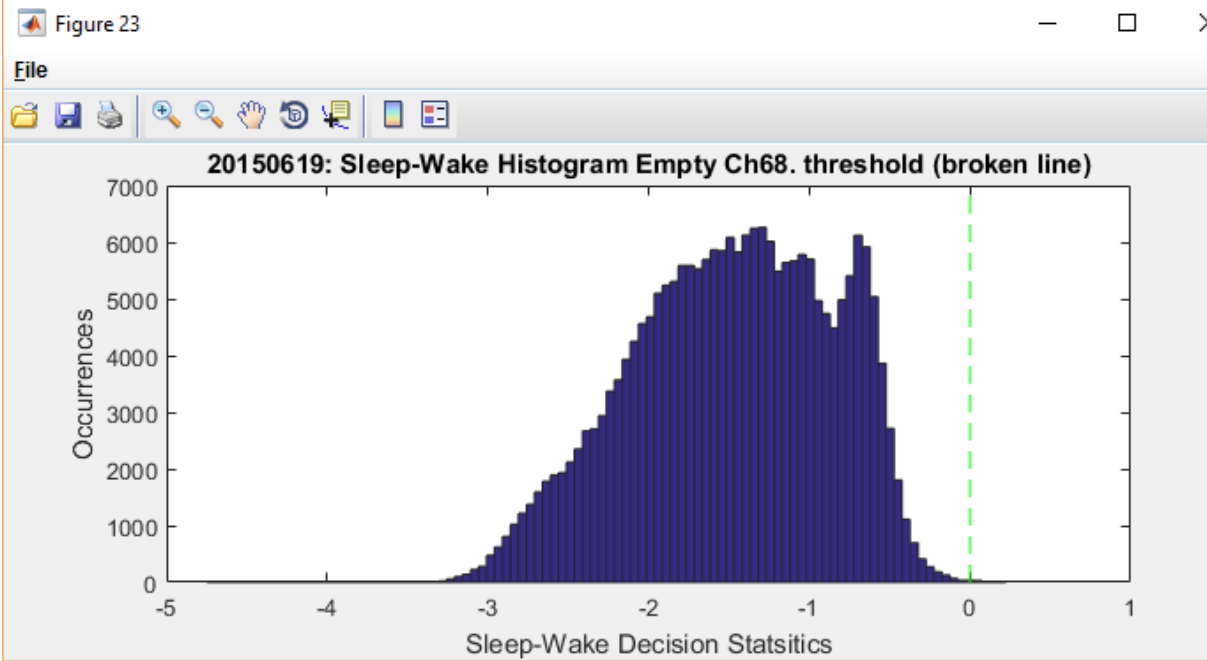


(b)

Figure 8. Examples of bimodal decision statistic distributions suggesting a good separation between sleep and wake activity.



(a)



(b)

Figure 9. Noise-only histograms (a) from a well isolate cage, and (b) from a cage with non-sleep like interference patterns (i.e. cage in the path wind from a vent or placed on a structure with vibrations).

2.3 Percent Daily Sleep

The *Percent Daily Sleep* button in the options menu (Fig. 2) computes the average percentage of time spent sleeping over and integer multiple of 24-hour periods for each channel and presents the results in the form of a bar plot as shown in Fig. 10. In a multiday recording, fractions of the 24-hour day are not used in the daily percentage 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. If partial days are used to compute the sleep percentage, the results can be biased depending on the light-dark state of the extra time added. The exception to these rules is when the recording time does not include 2 light onsets, in which case the whole file is used in computing the sleep percentage. If the first, partial day of the experiment is important, then the *Percent Wake Over Sliding Interval* button can be used to examine sleep-wake behavior as a function of time, or the *Hourly Sleep Percentage Export CSV* button can generate percent sleep statistics over user defined intervals from any beginning to any ending time within the recording period (see the sections on these options).

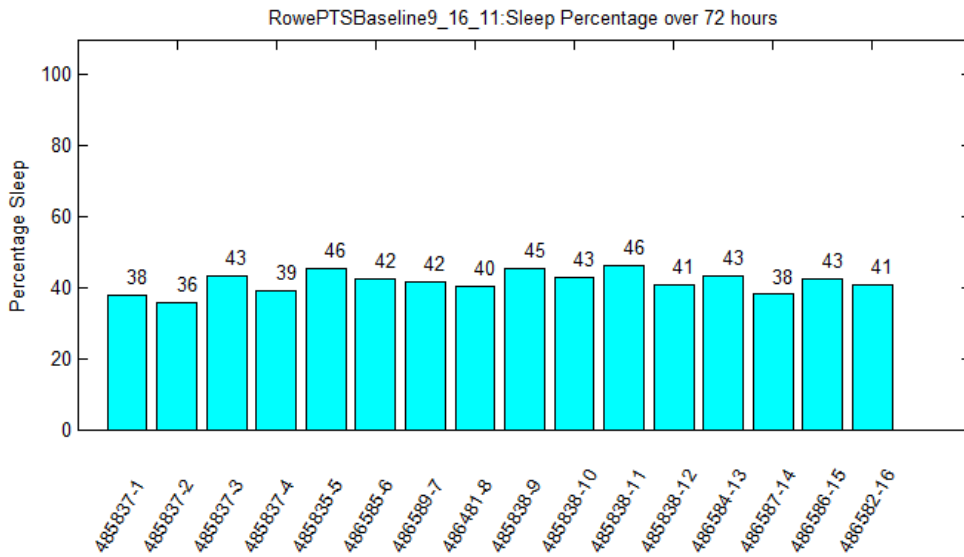


Figure 10. Daily sleep percentages averaged over 24 hour intervals.

2.4 Percent Sleep in Light/Dark

The *Percent Sleep in Light/Dark* button computes similar statistics to the ones shown in Fig. 10, except it separates the light and dark periods and presents side-by-side bar plots. For consistency with other plots, all data before the first light onset are excluded and the computation starts with the first light onset and ends at the last light onset. This way only full light and dark cycles are included to prevent a partial day from biasing the results. If 2 light onset times are not included in

the recording, then no plots will be created. If the partial days are important for this analysis, then the *Percent Wake Over Sliding Interval* button can be used to graphically examine sleep-wake behavior as a function of time, or the *Hourly Sleep Percentage Export CSV* button can generate percent sleep statistics over user defined intervals from any beginning to any ending time within the recording period (see the sections on these options for more detail).

An example for the same data used in Fig. 10 is presented in Fig. 11. Note the nocturnal pattern when comparing light (daytime) and dark (nighttime) bars, showing about double the sleep time in the light phase.

If unusual or interesting patterns are observed, it is a good idea to look at the decision statistics, sensor signal data (if available) and/or histograms to ensure the patterns make sense, and data were not affected by an unexpected event (deceased mouse, water bottle leakage, sensor/amp failure, inaccurate light-dark times in the room, daylight saving time changes during experiment, etc.).

The dark and light onset times are entered before data collection begins as part of the setup protocol in the *PiezoSleep* recording software. These are stored in the data file headers and extracted when the file is opened in subsequent programs. In the case of errors, however, they can also be changed in the *sleepstats* options menu. The *Change Processing Options* button presents a window where new times can be entered. The change options are explained in more detail in the *Change Processing Options* Section.

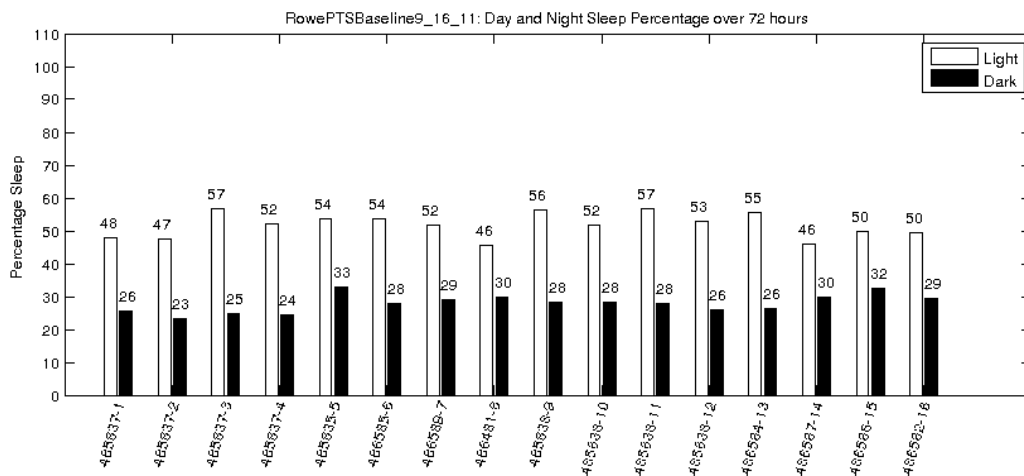


Figure 11. Average sleep percentage by channel for all time segments corresponding to a light environment (light bars) and a dark environment (dark bars).

2.5 Percent Wake over Sliding Interval

The *Percent Wake over Sliding Interval* menu option (Fig. 2) presents a window for selecting an individual animal and plotting the percent wake time over a sequence of time intervals that are incrementally advanced (sliding window) over the entire data segment. This is related to activity in the sense that the mouse is awake and engaged in either very low or high levels of activity. Equal weight is given for each 2-second interval independent of whether the animal was resting or running the cage. The plot indicates the percentage of time engaged in any kind of activity associated with a wake state.

An example wake activity plot is shown in Fig. 12. These plots are created by computing wake percentages over the interval lengths (default of 2 hours) that are incrementally advanced (default of 0.2 hours) over the whole recording. The percentage is assigned to the time point corresponding to the midpoint of the interval. Note in the menu bar at the top of the figure window there is an *Export CSV* option. If this is selected, a window will appear with a text box with a default filename to store the data, which can be modified if desired. The file will be saved in the same directory where the feature vector file is located. The file will contain the values of the percent wake state curve. The format of the output is shown in Table 1.

Table 1. CSV file for each animal, percent wake at timestamps in hours (left column) of the interval center, and light phase indicator (0 light, 1 dark)

19-Jun-15	Percent Wake	light phase
9.22	99.44	0
9.42	99.12	0
9.62	99.25	0
9.82	98.99	0
10.02	99.11	0

The last column denotes the state of the light/dark phase of the room based on the light and dark onset times provided when the experiment started. A 0 indicates light and 1 indicates dark. This can be further analyzed with a spreadsheet program. In addition to plotting individual mice, there is an option to plot all (generating a sequence of figures) as well as outputting the wake-state percentages for all mice to a single CSV file.

Examples of statistics that can be computed from this type of profile are annotated on the plot. One default statistic is the first significant wake-state percentage peak after dark onset (denoted by the red vertical broken line), with text indicating the time relative to dark onset. This value was computed from a 3 hour sliding window to generate a smoother curve for more stable estimates. This is why the red line does not necessarily intersect the peak of the 2-hour sliding window. Any peak within 5% of the maximum is considered significant and the significant peak closest to the dark onset is selected. The second default statistic, the Diurnal Wake Ratio (DWR), quantifies the strength of the nocturnal pattern. This number is the ratio of the maximum wake state percentage in the dark phase to the minimum wake state percentage in the light phase. These statistics are

computed starting at the first light onset after the experiment begins and are computed for every subsequent light-dark phase pair.

Custom statistics similar to these can be created by selecting a desired interval and increment for computing the wake percentages from the exported CSV files. The time interval and increment values can be changed from their default values using the *Change Processing Options* menu item. These values control the resolution of the wake-state percentage plots. The smaller the window, the more likely narrow intervals of wake will be captured on the plot. Broader time intervals tend to smooth-out/reduce short duration wake bouts. However, the larger the interval, the smoother the wake activity plot will appear. The *Change Processing Options* button presents a window where a different interval length and increment can be entered as well as the increment. This is explained in more detail in the *Change Processing Options* Section.

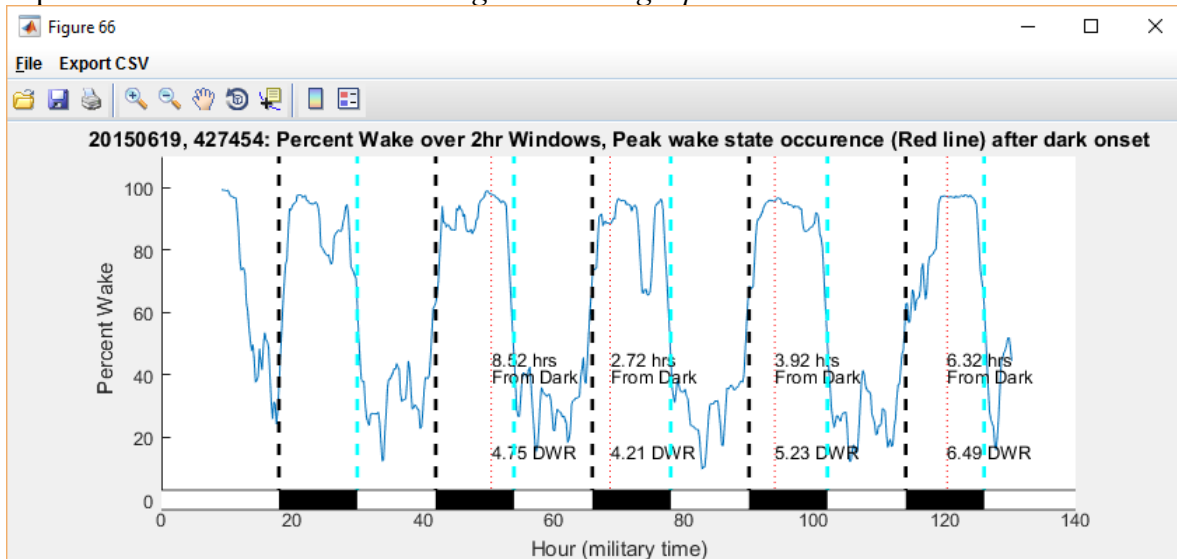


Figure 12. Wake activity plot showing percent wake averaged over a 2-hour sliding interval. The first dominant peak wake-state percentage after dark onset is denoted by the red broken vertical line. Text denoting this time interval is written on the graph. The Diurnal Wake Ratio (DWR) is also written on the graph. Menu item *Export CSV* is available to export the values of the graph to a CSV file (format of output shown in Table 1).

2.6 Sleep Bout Length Histograms

The *Sleep Bout Length Histograms* option (Fig. 2) computes a histogram of sleep bout lengths. Once this option is selected, another menu appears, shown in Fig. 13, to further refine the options. The histograms can be computed over the total period or separately for the light and dark periods (similar to the segmentations for the percent sleep statistics). As in the previous graphic statistic displays, the bouts are computed for all days between the first and last light onset. If 2 light onsets are not computed in the recording, then this graphic cannot be generated.

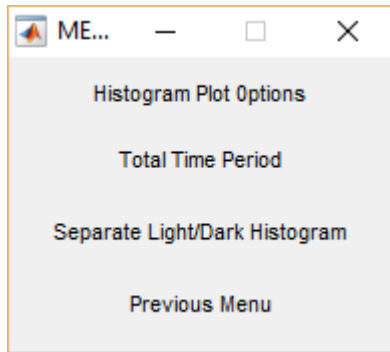


Figure 13. Sleep bout length histogram options menu.

Ideally, sleep interval histograms count contiguous intervals of detected sleep states to estimate a bout length, and these are binned over logarithmically scaled intervals (doubles previous interval). However, the occurrence of isolated instances of stirring or twitching during sleep bouts will typically cause wake detection disrupting a sleep bout. However, for some sleep bout studies, these values are ignored since the mouse returns to sleep or were never fully aroused from sleep. For this reason, *sleepstats* has a *minimum sleep bout length* parameter that allows the program to ignore short arousals in computing bout lengths. This also makes the bout estimate more robust against isolated errors in the sleep-wake classification. A bout length will only be terminated when an interval equal to the minimum sleep bout length has less than 50% sleep. So, any interval of this length with 50% or more sleep instances, will be counted as a sleep interval. Therefore, no sleep interval less than the minimum sleep bout length occurs in the bout estimation process. This interval will increase as this minimum bout length window is slid along every 2 seconds until an interval includes less than 50% sleep. This ends the sleep bout which is from the beginning edge of the first window to the end edge of the last. Since the program operates on 2 second detections, setting the minimum bout length to 2 seconds enables the program to operate at its maximum resolution. However, with an expected error rate of 5% to 10%, operating at the highest resolution may result in the bout length statistics reflecting that error. If the minimum bout length is set to 30 seconds (15 detections in the intervals), it is highly unlikely that 8 errors will occur in that interval to end the current bout computation or initiate a new one. The probability of a bout ending or continuing in error (for a high expected error probability of 10%), is 3.1×10^{-5} , which comes to about 1 error every 16 hours). The default time is set to 30 seconds but can be changed using the *change minimum sleep bout length threshold* in the change parameters option.

Figure 14 shows an example of the sleep bout length histogram for a full data recording (x-axis has a logarithmic octave scaling of the minimum bout length (doubling) and y-axis is a percentage of total sleep time the mouse spent in each bout range). By default, sleep bouts are expressed as percent of sleep time for each bout length interval. The mean bout interval length of 369.3 and standard deviation of 77.6 seconds are presented in the figure title. Figure 15 shows sleep histograms for the same data in Fig. 14, except the light and dark phases are separated. Note the light phase sleep bouts are on average longer than the dark phase sleep bouts. The last bin includes percent time spent in all bout lengths greater than 3048 seconds.

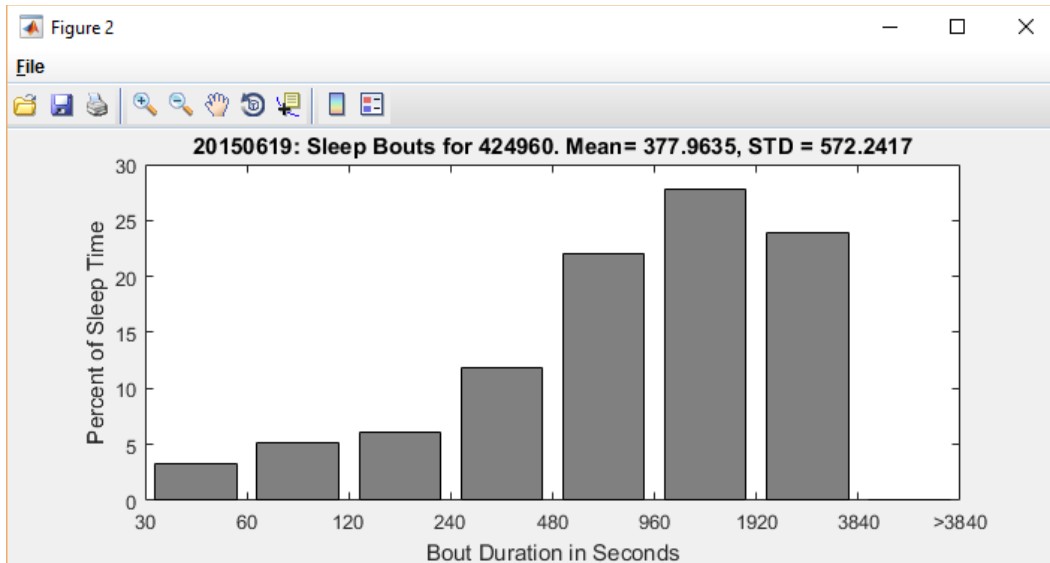
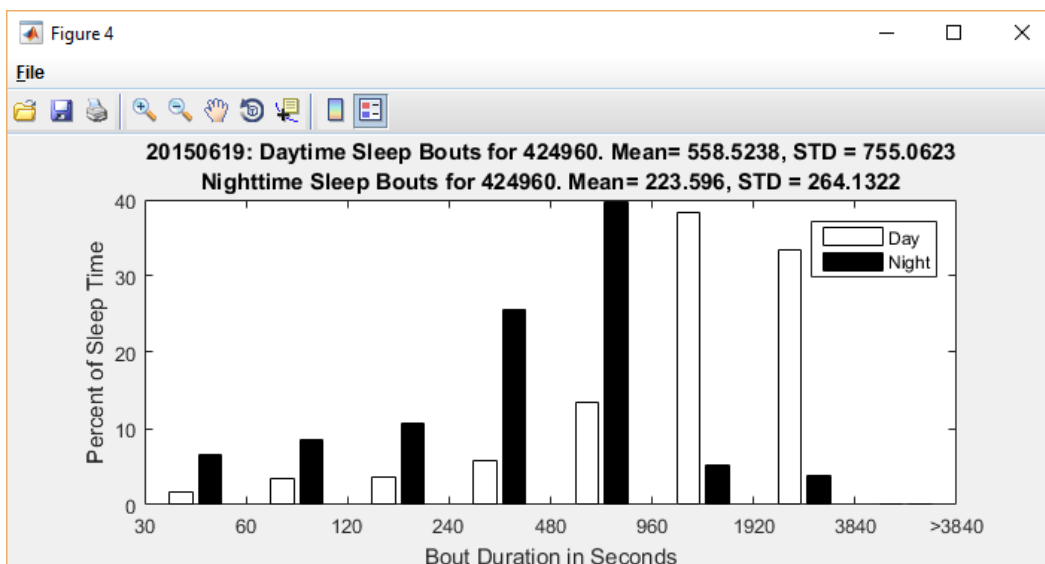


Figure 14. Sleep-bout length histogram for total recording.

2.7 The normalization of the bin values can be modified in the Change Processing Options menu item. The default is to normalize over the particular state (either sleep or wake). In this case, the bin values will always add up to 100%. The other option is to normalize over total time. With this option, the bins will add up close to the total sleep time. This total may not agree with total percent daily sleep because the operations with the minimum bout length causes a rounding up of sleep intervals. If the bout length is set to 2 seconds then there will be agreement with these numbers. The third normalization option indicates the number of intervals detected in each bin range (Y axis is changed from percent of sleep time to number of bouts).



2.8 Figure 15. Sleep-interval histogram separated into light and dark phases.

2.9 Wake Bout Length Histograms

2.10 The option for plotting the wake bout lengths is completely analogous to the sleep bouts, except the 2 states are reversed.

2.11 Daily Sleep Stats Export CSV

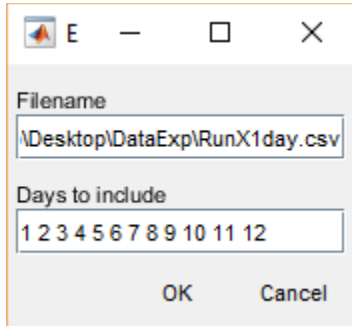


Figure 16. Prompt to save CSV file with daily statistics for a 12 day recording.

This option computes common sleep statistics over 24 hour intervals, starting with the first light onset, and saves them in CSV format. A prompt allows the file name or included days to be modified, as shown in Fig. 16. The default output file name is the data file base name with a .csv extension, and the default days include all full days of recording in the experiment. The format of the CSV file and the statistics are described in this section. An example of a CSV file is shown in Fig. 17. The first row consists of the header labels, which are in bold and all capital letters, described below:

MOUSE_ID – label to identify mouse in experiment.

TESTDATE – date of period from which the statistics for the first day were computed. This is the first light onset following the start of data recording. The TESTDATE is typically not the same as the day the recording started, since most recordings tend to start in a light phase time period.

For the following labels, the # symbol represents the day of the experiment relative to the *start of recording* (i.e. 1, 2, 3, 4 ...). The days selected do not need to be consecutive.

PERCENTSLEEPTOT# – percent of time in sleep state for 24 hour period.

PERCENTSLEEPNIGHT# – percent of time in sleep state during dark phase.

PERCENTSLEEPDAY# – percent of time in sleep state during light phase.

BOUTLENGHTTOT# – average sleep bout length over 24 hour period in seconds.

BOUTLENGHTNITE# – average sleep bout length during dark phase in seconds.

BOUTLENGHTDAY# – average sleep bout length during light phase in seconds.

DIURNAL WAKE RATIO# - diurnal wake ratio for the given day (unitless, same as computed for percent wake over sliding window plots).

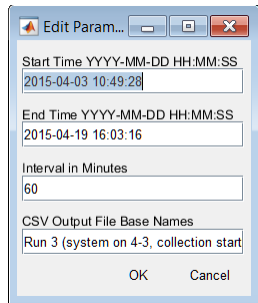
PEAKACT# – time in hours relative to dark onset when effective maximum percent wake-state activity plot occurs in the dark phase for a given day (same as computed for percent wake over sliding window plots).

	A	B	C	D	E	F
1	MOUSE_ID	TESTDATE	PERCENTSLEEPTOT4	PERCENTSLEEPSNIGHT4	PERCENTSLEEPDAY4	BOUTLENGHTTOT
2	BX1	7/1/2014	45.08	20.28	69.88	614
3	BX2	7/1/2014	39.23	22	56.45	443.2
4	BX3	7/1/2014	43.66	16.33	70.99	409.1
5	BX4	7/1/2014	40.59	24.19	56.98	461.5
6	BL1	7/1/2014	36.39	16.15	56.63	441
7	BL2	7/1/2014	34.61	19.6	49.63	789.2
8	BL3	7/1/2014	34.93	26.08	43.77	277.0
9	BL4	7/1/2014	36.83	23.29	50.36	246.2
10						
11						
12						

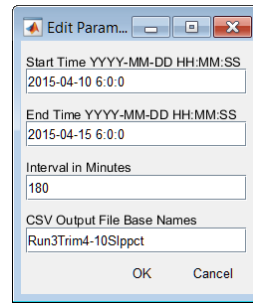
Figure 17. CSV file containing sleep statistics imported into Excel, where days requested started with day 4 of the experiment

2.12 Hourly Sleep Percentage Export CSV

The *Hourly Sleep Percentage Export CSV* button in the options menu (Fig. 2) creates a CSV file with percent sleep computed over user defined intervals (1 hour is the default interval). The first column consists of time stamps corresponding to a sequence of the start times for each interval. If this option is selected, a text box appears with the start and stop time of the complete file and the last saved interval. Examples of these text boxes are shown in Figs. 18(a) and (b). The text box can be edited to create a custom sequence. An example of the CSV file is shown in Fig. 18a, imported into Microsoft Excel. The first row contains the date the experiment began followed by the animal IDs. The time stamps begin with the actual start time of the experiment in military format, so the time stamp reflects the time of day at any point in the experiment. The default interval for calculating the sleep percentage is 60 min (1 h). This can be changed through the *Change Processing Options* menu. The default CSV file name is the same as the base name of the open *FeatVecfb* file and appended with the string *slppcnt*.



(a)



(b)

	A	B	C	D	E	F	G	H	I	J	K
1		CD1F3121	CD1F3122	CD1F3123	CD1F3124	BL6F311	BL6F312	BL6F313	BL6F314		
2	4/10/2015 6:00	59.04	37.43	8.74	4.81	45.85	40.87	33.8	40.39		
3	4/10/2015 9:00	21.74	27.52	45.76	51.39	36.48	49.91	48.94	45.13		
4	4/10/2015 12:00	64.28	80.94	74.52	84.83	59.5	42.02	63.39	48.43		
5	4/10/2015 15:00	72.46	70.44	80.26	78.39	63.63	56.22	62.93	59.11		
6	4/10/2015 18:00	73.13	62.5	61.28	74.87	51.54	64.33	67.28	54.59		
7	4/10/2015 21:00	44.61	23.06	41.39	16.93	18.78	33.19	17.11	27.28		
8	4/11/2015 0:00	36.06	26.74	23.41	38.48	14.74	32.31	10.78	11.02		
9	4/11/2015 3:00	3.65	8.07	5.09	50.63	57.06	28.3	23.09	25.17		
10	4/11/2015 6:00	50.85	21.93	8.67	8.63	42.39	46.09	20.02	18.85		
11	4/11/2015 9:00	37.74	39.26	61.69	66.76	32.54	52.87	54.35	59.94		
12	4/11/2015 12:00	15.83	35.59	32.89	52.37	31.67	29.06	26.07	30.69		
13	4/11/2015 15:00	75.52	83.3	78.11	81.74	62.07	57.94	61.76	67.52		
14	4/11/2015 18:00	71.35	69.2	74	73.78	66.93	60.06	62.83	55.15		
15	4/11/2015 21:00	47.85	38.85	44.07	24.46	27.24	34.56	27.57	32.09		

(c)

Figure 18. a) Initial menu over entire time range and default values. b) Edited parameters for user specific output csv file. Analysis interval increased to 3 hours and time range trimmed to 5 days starting at 6am on April 10. CSV file saved in the same directory as the Feature Vector File opened in the analysis session. c) Example of CSV output resulting from the menu parameters in b).

2.13 Hourly Sleep Bout Stats Export CSV

This option works similar to the exporting of sleep percentages described in the previous section. It can export several statistic files that describe sleep bout behavior. These sleep bout statistics include the histogram bin valves, the mean, median, standard deviation, 10th percentile, and 90th percentile sleep bout lengths in units of seconds. The percentage of sleep bouts greater than a user defined threshold (default is 256 seconds) is also available. This threshold can be changed under

the *Change Processing Options* button and is useful for exploring the likelihood of the less frequent longer sleep or wake bouts.

Since not all these statistics may be relevant to a study, a prompt appears to select the statistics files to output. The sub menu appears, shown in Fig. 19(a), where clicking on the statistic name will toggle the output files to selected and deselected, as shown in Fig. 19(b) for the case where the histogram and mean are selected. Click on the *done* test to proceed once the selection is made. For each item selected, a spreadsheet is generated with the corresponding statistics for each time interval and each animal. The only exception to this is the histogram, which outputs histogram values for each bin interval for each animal for the entire time range specified computed from the very beginning to the very end of the time range specified by the user. A window similar to those shown in Figs 18 a and b is generated when this option is first selected. The time range can be modified through these text dialog boxes.

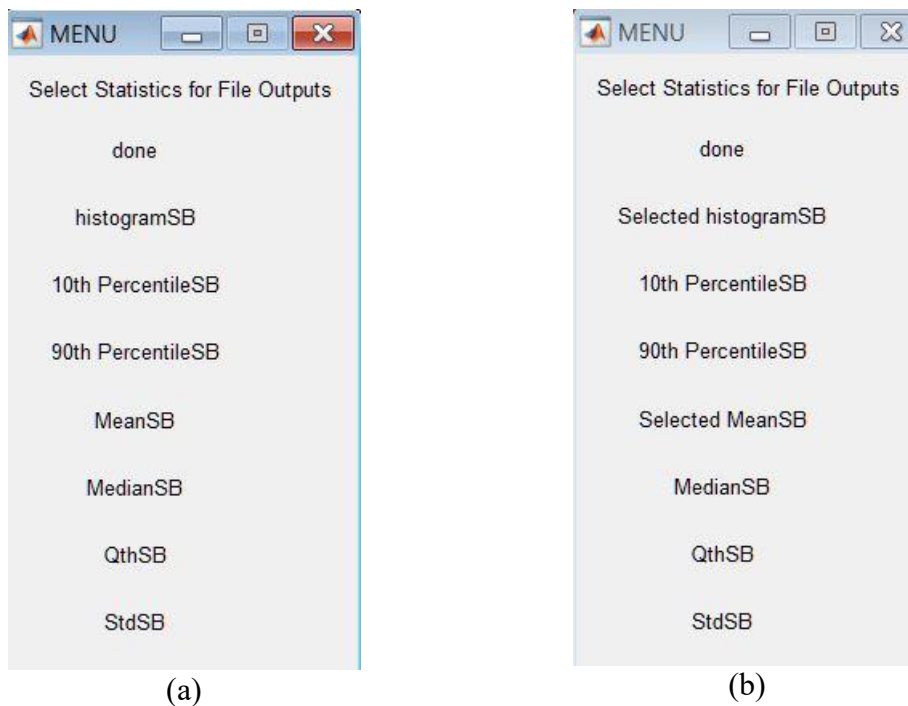


Figure 19. Example of a menu to select output files with wake bout statistics. Clicking the mouse on the statistic name toggles from select to deselect. Click done when selections are made. If no files are selected, no files will be output. (a) Initial Menu with nothing selected. (b) Menu appearance after the histogram and mean have been selected for output.

The exported CSV files are distinguished by strings appended to the base name. An example of a set of files exported by this program is shown in Fig. 19, where the base name of the file was *RowePTSPost-Injury9_23_11*. The appended strings correspond to each statistic as follows:

Sleep Bout Length Statistic	Units	Appended String Sleep	Appended String Wake
Histogram	Percent Time	_histogramSB	_histogramWB

Mean	Seconds	_MeanSB	_MeanWB
Median	Seconds	_MedianSB	_MedianWB
Standard Deviation	Seconds	_StdSB	_StdWB
10th percentile	Seconds	_10ptileSB	_10ptileWB
90th percentile	Seconds	_90ptileSB	_90ptileWB
Percent Less than Threshold Q	Percentage [0 1]	_QthSB	_QthWB

2.14 If an interval does not include at least one sleep bout, a NaN (not a number) is output. A zero is not appropriate in this case, since the number of bouts is not being computed, it is computing bout length statistics in seconds. So, the NaN represents that a length could not be computed because no bout existed to measure. Many spreadsheets and mathematical programs have functions that will automatically ignore NaN values when computing statistics, such as mean and standard deviations. A zero value would erroneously bias this computation low.

2.15 Hourly Wake Bout Stats Export CSV

2.16 The option for exporting Hourly Wake Bout Stats is completely analogous to the Hourly Sleep Bout Stats, except the 2 states are reversed.



Figure 20. Options to change default parameters

2.17 Change Processing Options:

Many of the plots and statistics computed for the options described in the previous sections are dependent on specific parameters involved in the computations. The user can change some of these values through this menu. This section explains how these parameters impact the statistics and graphs, and describes the procedure for changing the values.

If the *Change Processing Options* is selected from the options menu (Fig. 2), the submenu shown in Fig. 20 will appear. Some of the parameters were created at the time the data was collected and saved in the header of the data file, such as the *Light/Dark Times*, and others are set as defaults in the *sleepstats* program. The parameters can be changed and saved to a companion database file with a *.mat* extension (if *Save Changes* is selected). This way, the next time the *FeatVecfb* file is opened, the *.mat* file will be loaded with the last saved parameters. If the *.mat* file is deleted or not in the same directory as the *FeatVecfb* file (with the same base name), the parameters will revert to their default values and a new *mat* file will be created.

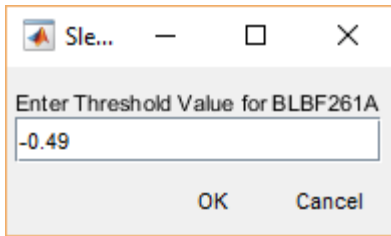


Figure 21. Text box window for changing the decision threshold.

If the *Change Sleep/Wake Decision Threshold* option is selected, a window will appear prompting the user to select a channel (similar to the one in Fig. 3b). Once a channel is selected, the text box shown in Fig. 21 will appear. The number in the text field is the current threshold value. A new value can be entered and will replace the original value in subsequent computations and plots. The *Save Changes* option will save the new value to the *.mat* file, if selected. Otherwise the original value will be used after the file is closed and reopened.

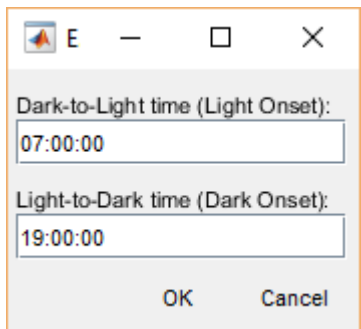


Figure 22. Text box window for changing light and dark transition times.

The *Change Light/Dark Times* option is used to change the light and dark onset times of the recording. These parameters should have been set when the recording was initiated. However, the user may want to change them through the dialog box shown in Fig. 22. This may be the case if the original values were determined to be incorrect, or if the user wanted to explore different ways to segment the data on a daily cycle. The times are given in military format relative to the 24 hour daily cycle. If a change is made to these parameters, it will apply to all subsequent results (*i.e.* it will not update older figures). The *Save Changes* option will save the new values to the *.mat* file for recall every time this file is opened (the defaults will not be used). These parameters will affect all light/dark phase statistics, such as percent sleep in light and dark.

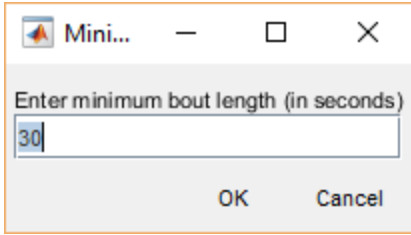
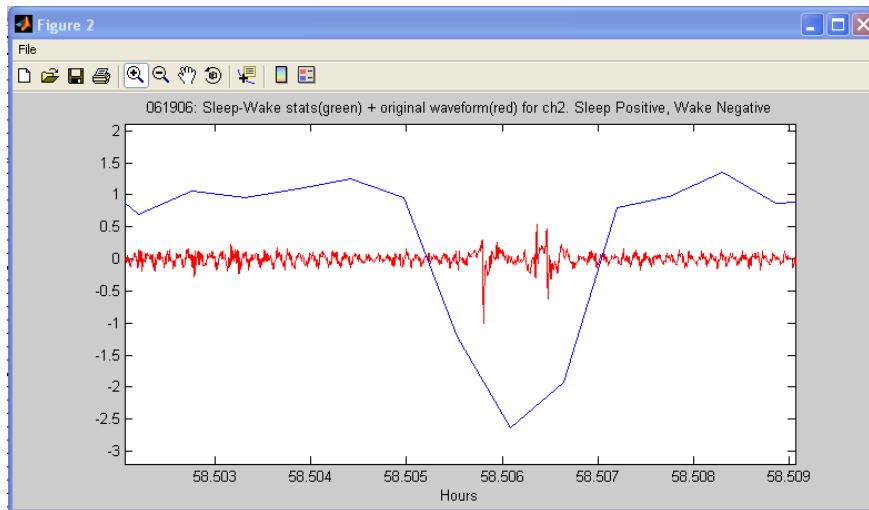


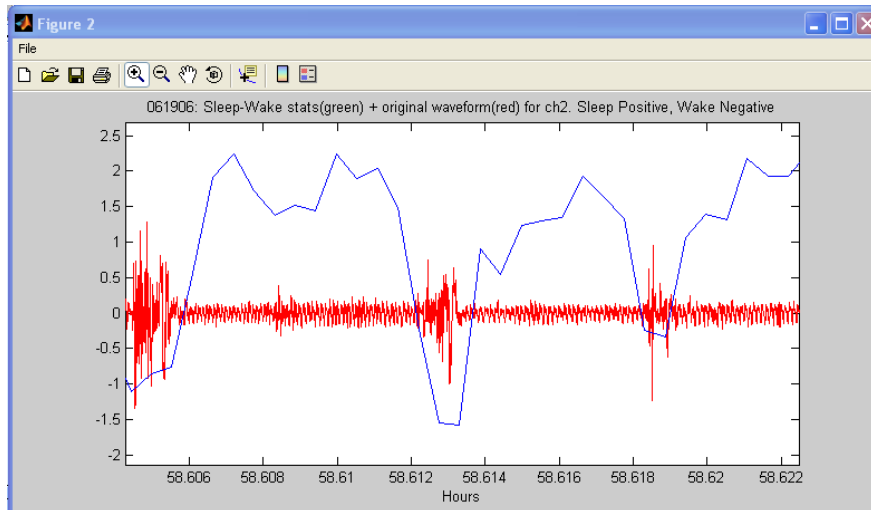
Figure 23. Text box for changing the sleep movement threshold.

The *Change Minimum Sleep/wake Bout Length* option only affects the default values used for results related to the sleep bout lengths. For the CSV output of bout length statistics, this value can also be changed when selecting the export parameters in the text box that appears. However, the next time it is selected, it will return to this default value. Time intervals with greater than 50 percent sleep will be marked as sleep, and will not interrupt or end a sleep bout computation. Only when an interval contains 50% or greater of wake detections will the bout come to an end. Figure 23 shows an example of the text box presented for changing this parameter. The current value is initially shown in the box (where the default value is 30 seconds). The smallest interval that can be selected is 2 seconds, which is the maximum resolution of the decision algorithm. In general, increasing the minimum bout length threshold increases the number of longer sleep bout lengths. This parameter is also applied to wake bouts.

Figure 24 shows examples of wake detections that would not end a sleep bout because they are less than 50% of a 30 second window. Figure 24(a) is about 30 seconds with 3 wake detections from a brief stirring (wake is detected when the blue line falls below zero). The other 12 detections surrounding it are sleep. If the window was set to 6 seconds, this would end the sleep bout. A similar situation is observed in 24(b), where 60 seconds of data are shown. As a 30 second interval is slid (every 2 seconds) over the sequence, the wake detections never dominate the interval.



(a)



(b)

Figure 24. Examples of short movements detected during longer sleep bouts are classified as wake periods (a) 25 second segment with a 6 second wake interruption between sleep (b) 64 second segment with a couple of 4 second wake interruptions between sleep.

The option to *Change Percent Wake Sliding Interval Parameters* affects the wake percentage plots and the peak wake percentage after dark onset times and DWR statistics. The wake percentage plots are generated by computing the percentage of wake states over a sliding window. The default value for this window is 2 hours and the increment between windows is 0.2 hours. The percentage of wake states over each window is assigned a time point corresponding to the center of the window. Figure 25 shows the dialog box for changing the window length over which the wake percentages are computed.

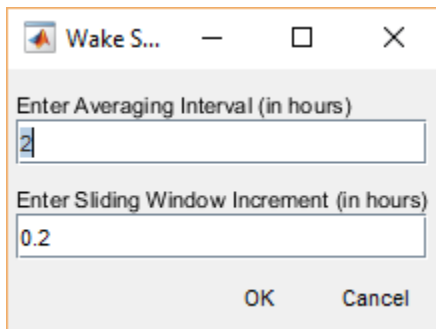


Figure 25. Dialog box for changing the wake-activity sliding window length.

Figures 26 and 27 show examples of how window length affects the wake-state percentage plot. Figure 26 is a set of sleep-wake statistics collected over 3 days. The sliding window computes the percentage of time the mouse was in the wake state in this interval. Examples of 1, 2, and 4 hour windows on these data are presented in Figure 27. Note how a smaller window follows the transitions more closely and reveals transient spikes, while a larger window smooths out (averages) the local transitions and reveals larger scale trends.

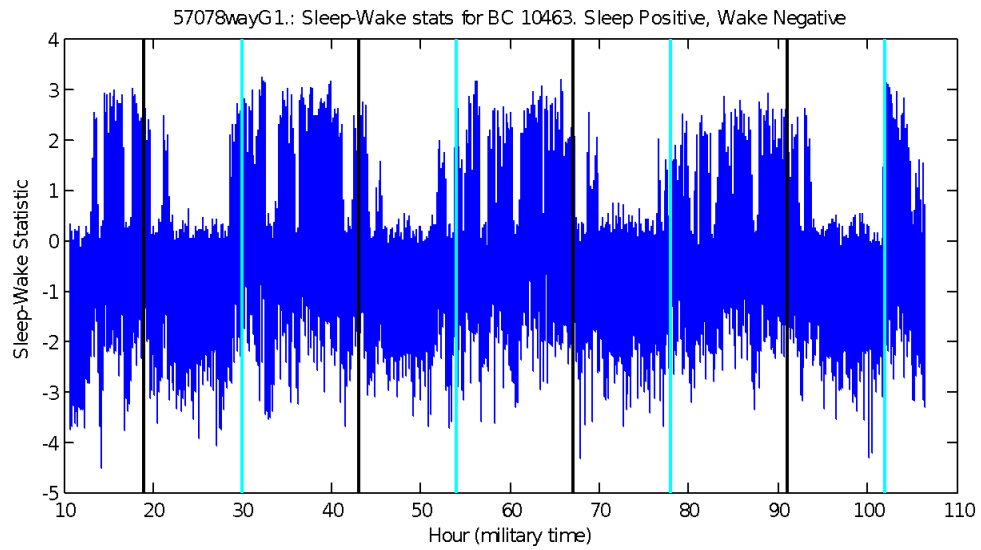
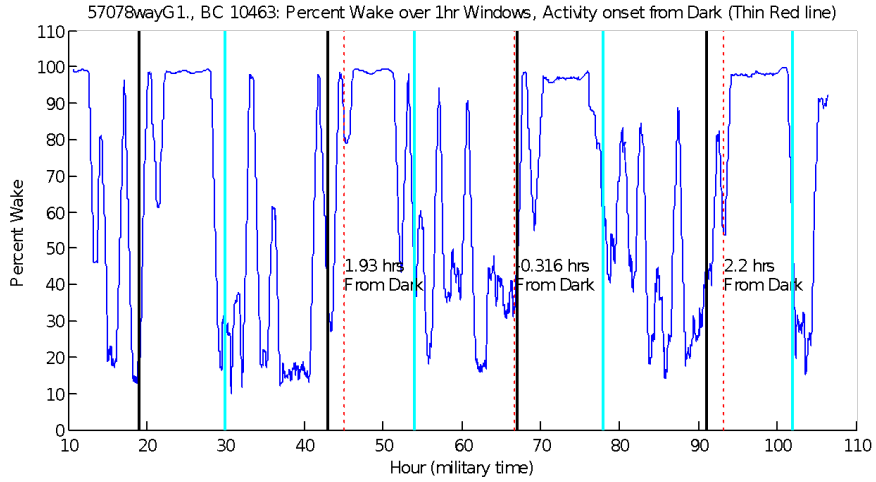
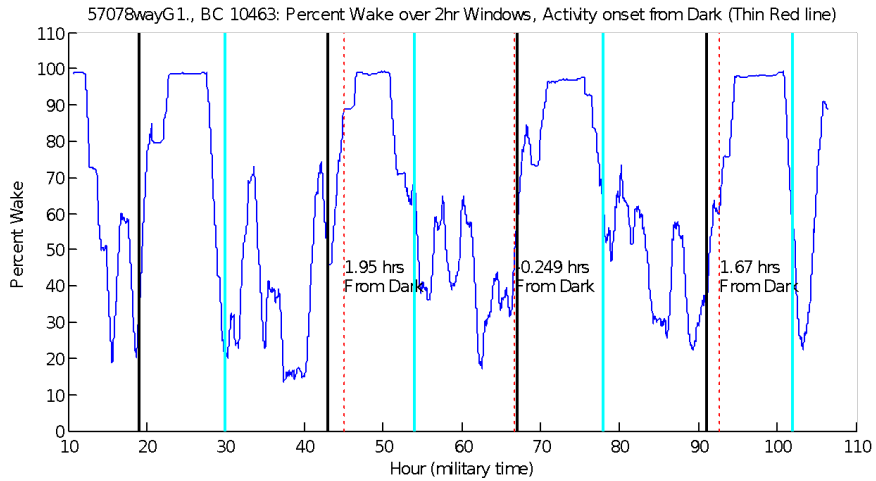


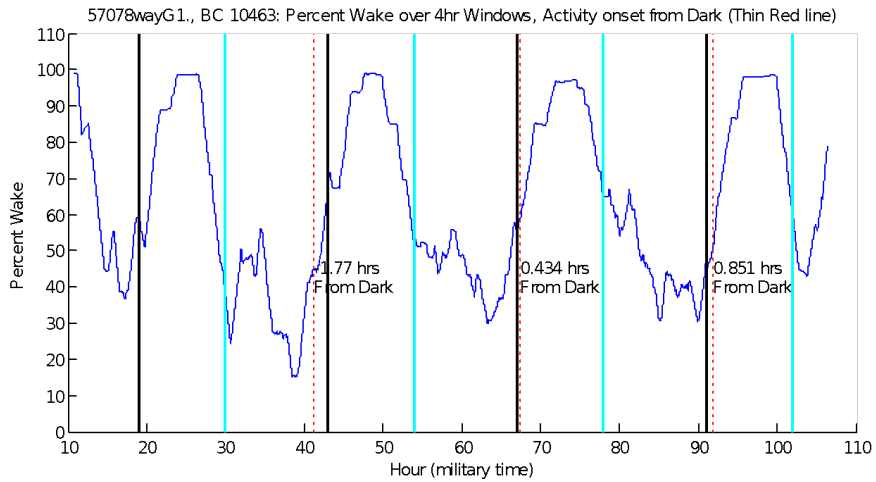
Figure 26. Sleep-wake statistics corresponding to wake activity plots in Fig. 27. Sleep-wake decision threshold at 0.



(a)



(b)



(c)

Figure 27. Percent wake state plots demonstrating the impact of sliding window length on original sleep-wake statistics shown in Fig. 26. Window lengths are (a) 1 hour (b) 2 hours (c) 4 hours.

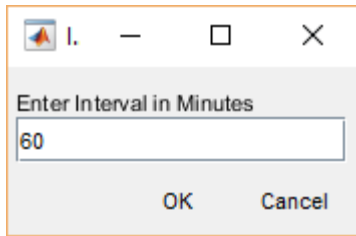


Figure 29. Text dialog box for changing the default intervals used in exporting sleep percentages and bout length statistics.

The option to *Change Interval for Sleep Percent CSV Export* only impacts the intervals for exporting the “hourly” percent sleep and bout length statistics, which are set to 60 minutes by default. This can be changed through the dialog box this option presents, shown in Fig. 29.

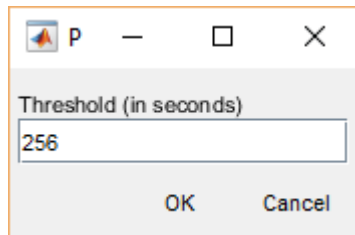


Figure 30. Dialog box for setting the threshold for computing percentage of sleep bout length greater than this value.

The *Change Bout Percentage Q Threshold* option changes the threshold on sleep and wake bout lengths for computing the percentage of bout lengths greater or equal to this value. This only affects the CSV bout length export file (QthSB or QthWB in Fig. 19). Figure 30 shows the dialog box indicating the default value of 256 seconds. This is particularly useful for exploring the events characterized by long bout lengths. If long bout length has significant meaning for your experiment, this statistic more directly characterizes low-probability events (i.e. in the tail-of-the curve) than the median or mean.

The *Change Sleep/Wake Bout Bin Normalization* option allows for different scales to be applied to the histograms of bout lengths. The normalization method is selected through the dialog window option shown in Fig. 31. The default option is *State Time*, which computes the amount of time spent sleeping in bout lengths that fall in each histogram bin range (or time spent awake for the wake bout option). These times are then divided by the total time for all sleep bouts. For this normalization the histogram bins add up to 100%. The other option, *Total Time*, also computes the time spent sleeping in each range of bout lengths, but divides by the total time in both states. For this normalization option the histogram bin will add up to the approximate percentage of time spent in the sleep state (as shown in the percent daily sleep). Because of the rounding up in

windows that have greater than 50% sleep, this total is typically greater than the percent daily sleep values computed over 2 second intervals. If the minimum bout length is set to 2 seconds, then these values will agree. Finally, the *None* option simply plots the number of bout lengths detected in each bin range. This option will also apply to the histogram values exported as CSV files.

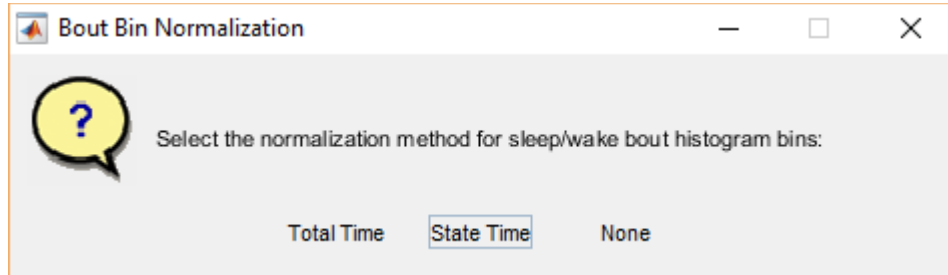


Figure 31. Dialog window to select normalization method for the bins in the bout length histograms.

The *Change Multi-Channel Plot Limit* option affects how the bar charts for percent sleep are segmented. The default value is 24, which means the bar chart will create a new figure once 24 channels are plotted. This prevents the chart from getting too crowded/busy. It only affects graphics for percent daily sleep.

Finally, the *Save Changes* option opens the *mat* file associated with the *FeatVecfb* file and saves these parameters, so the next time the *FeatVecfb* file is opened, the default values will reflect the last saved changes. For this to happen, a *mat* file with the same base name as the *FeatVecfb* file must be in the same directory. So if the data files are moved, make sure the *.mat* file is moved with them. If the *.mat* file is deleted, then the next time the data file is opened, the parameters will be restored to their original values. If this button is not selected before the current file is closed, the last saved values will be loaded the next time the *FeatVecfb* file is opened.

Appendix A: Manual Threshold Settings for Sleep Wake Decisions

The characterization of sleep and wake behavior from the rodent pressure signals involves **signal amplitude, quasi-periodicities, and regularity/consistency** of the signal amplitudes. Relatively low amplitudes imply smaller/weaker movements typical for sleep, 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 form a model for sleep-like and wake-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 from cases where the rodent 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. A1, where it is assumed the decision statistics are clustering around these 2 dominant behaviors.

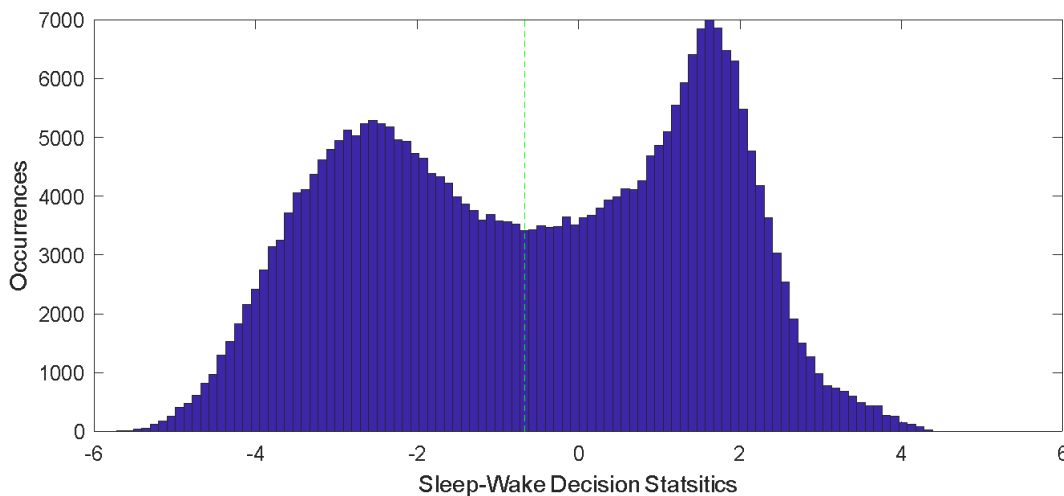


Figure A1. An example of a good bimodal distribution of decision statistics.

The vertical green line is the placement of the threshold. 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 side results from wake behavior. In many cases, the wake behavior is more varied and the peak may not be as distinct as the sleep behavior, shown in Fig. A2 below:

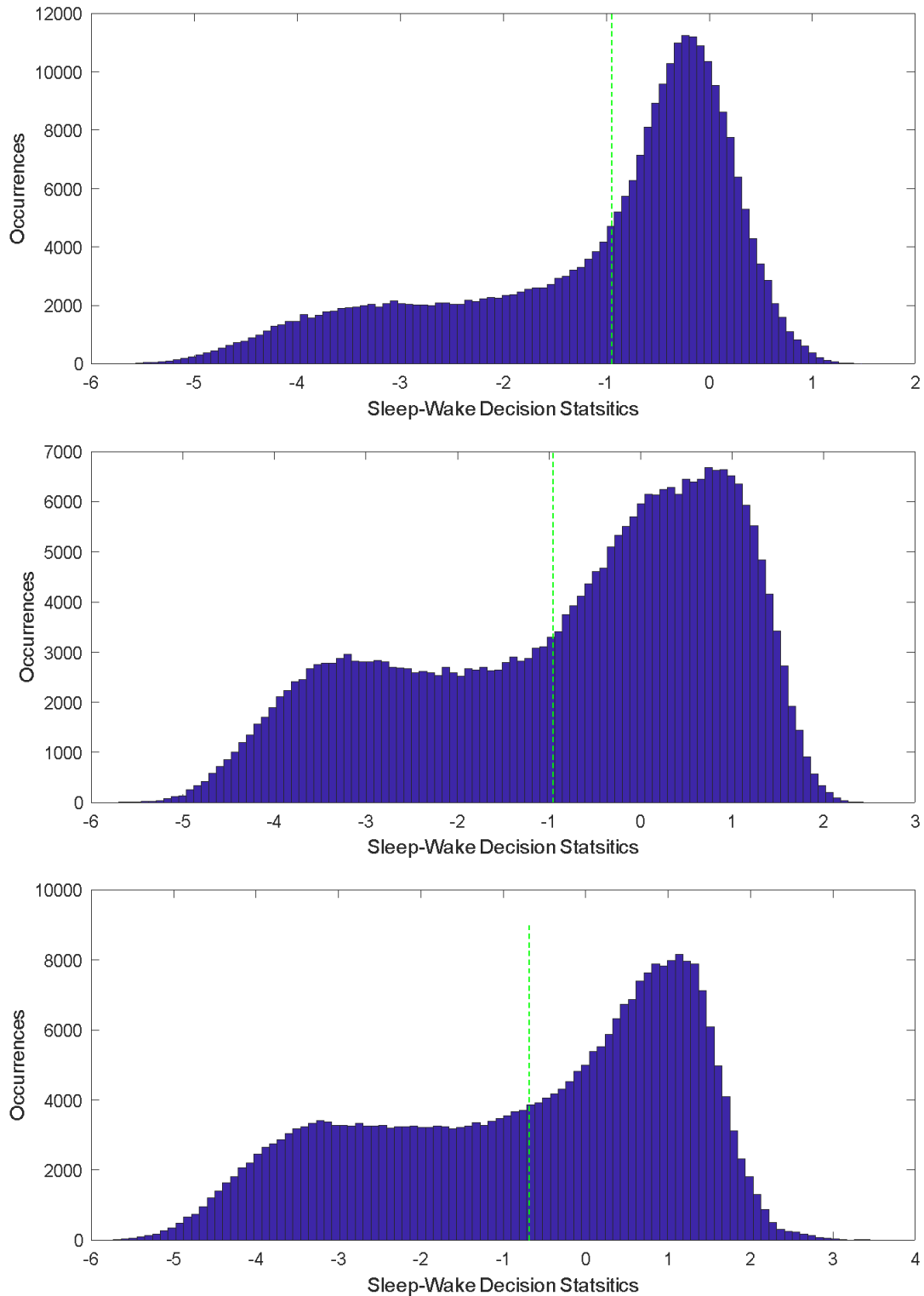
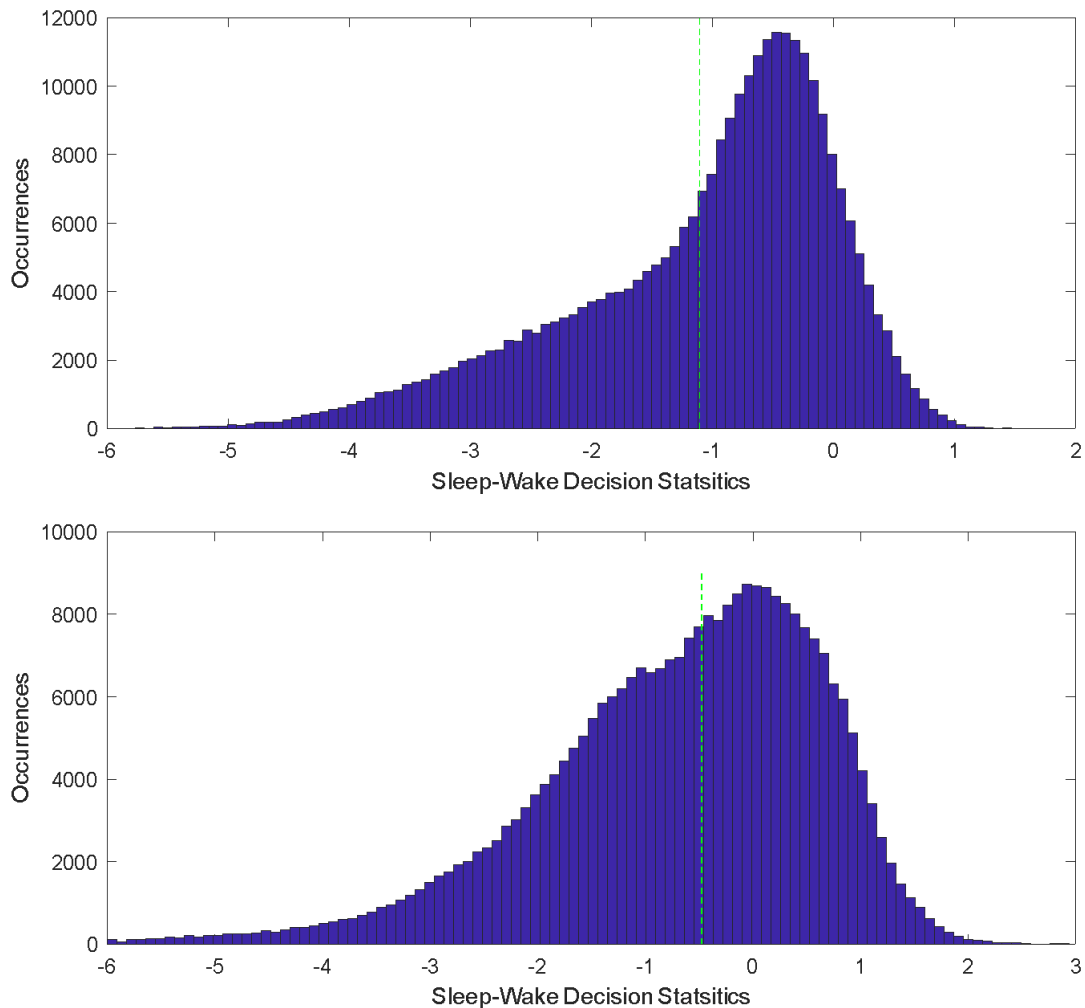


Figure A2. Examples of high variance in the bimodal distribution.

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) you will not need to make a threshold adjustment.

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, variations in 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 statistics, such that the sum of the resulting variances from both sides is minimized. This works in almost all cases, however in the cases of weak signals, larger noise levels, or unusual (illness) behaviors, the bimodal pattern can degrade. This can cause the algorithm to set the threshold in an unusual/wrong place. Examples of high noise recordings are shown in Fig. A3. The last figure is from an empty cage. Since there were no animal changing behaviors (just noise), it has a strong unimodal and symmetric shape.



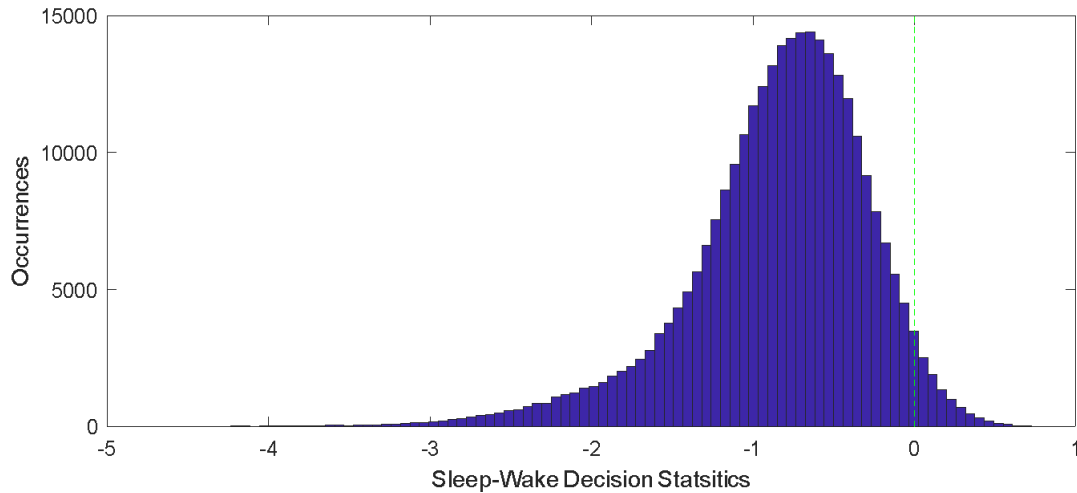


Figure A3. Examples of high noise statistics, the last being an empty cage (all noise).

When an extreme or unusual pattern is observed (i.e., odd-shaped decision statistic histogram or sleep percentages too high or too low) the pressure signal should be inspected for integrity. If a bad signal is observed (i.e., typical patterns of wake or sleep activity is not observed, or amplitudes too low or too high over all), 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 statistics if typical sleep and wake patterns can be seen in the cage floor pressure signal. This adjustment involves a level of subjectivity as to what pressure signals are *sleep-like*.

Below in Fig. A4 is an example of a case that showed high levels of sleep (80% to 90%). Note the decision statistics are all shifted high and the histogram is very narrow. This was a channel where the signal consistently gave low numbers, as if there was a bad sensor, amp, connection, or potentially a sick animal with limited movement.

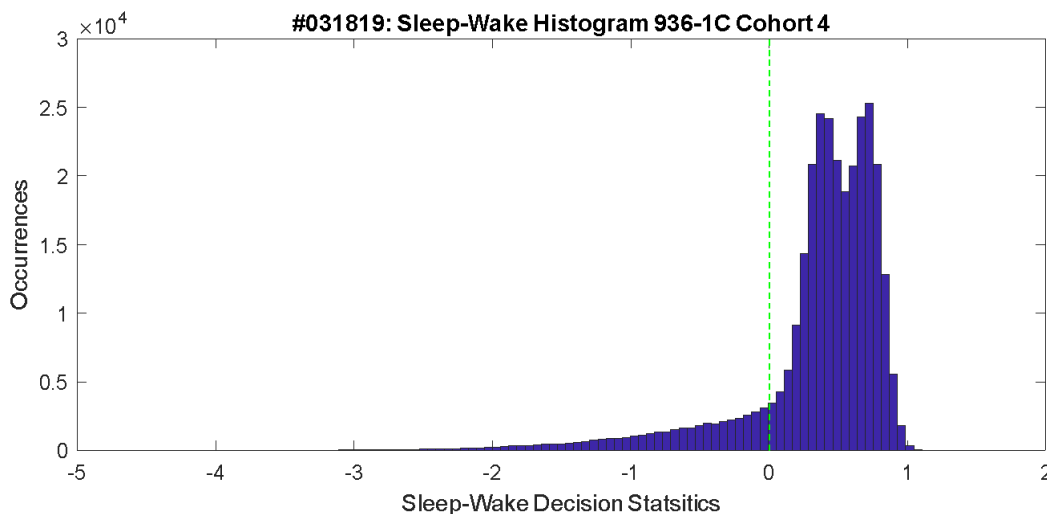


Figure A4. Example from a weaker than normal signal.

To provide more insight into what is happening here, use the first button in the main menu to plot the decision statistics (time series) for this animal. This results in the Fig. A5 below:

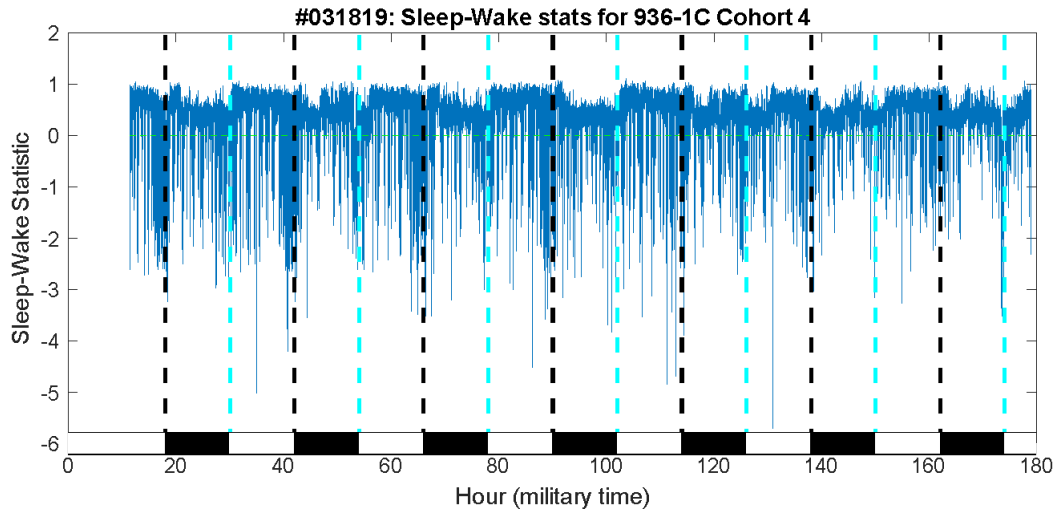


Figure A5. Time series decision statistic for the same animal as Fig. A4.

In the time series the green horizontal line is the threshold for the sleep and wake decisions. The histograms would result from projecting the data onto the y-axis, which represent the decision statistic values. Note a mild trend is observed with a dip in values during the dark phase (wake), and higher values in the light phase (sleep). This suggests putting the threshold between the clusters. If you zoom in on the above figure (Fig. A6) to a few hours around a transition. (In zoom mode, you can put the mouse pointer on the figure, hold down the left button and drag out an area to zoom into. Can also right click on the figure in zoom mode to get a menu to get an option to restore the figure to the original view).

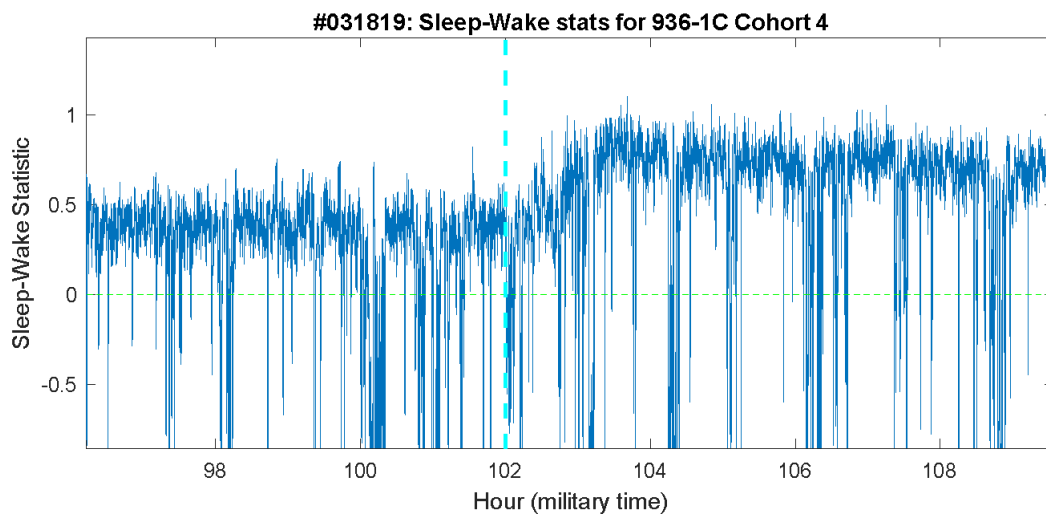


Figure A6. Zoomed statistic plot from Fig. A5.

If you now overlay the original pressure signal on this figure (Fig. A7), the source of the problem is observed. The amplitudes for activity patterns are relatively small (usually at least 40 mV), the signal is smaller than what we would typically measure. This could be the result of a bad sensor or a weak/sick mouse. You can zoom in on the low-level red line (note, the y-axis is now in 10^{-3} millivolts). 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 be.

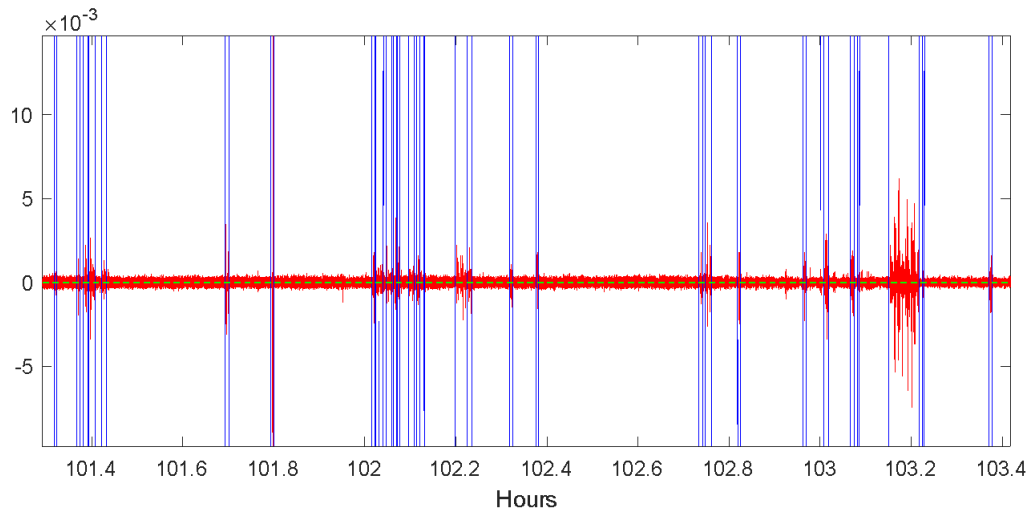


Figure A7. Decision statistic with pressure signal overlay

For this case, a threshold of about 0.4 or 0.5 would be appropriate. Zoom in on the histogram (Fig. A1.4) if you want to find the saddle point more precisely. To change the decision threshold for this animal, go to “change processing options” near the bottom of the main menu, and you will see a button to change sleep/wake decision threshold. Click on that then the animal ID you wish to change the threshold for, and it will apply the new threshold only for this session. If you want to save it for future times when this file is opened again, in that same sub-menu click “Save Changes”. This will save the threshold information in the mat file (a parameter file associated with the recorded feature vector file) and will apply it the next time that file is opened, provided the mat file has not been deleted or moved into a different directory.

Below in Fig. A8 another example of an outlier that indicated almost zero percent sleep. The histogram looks like a noise only recording:

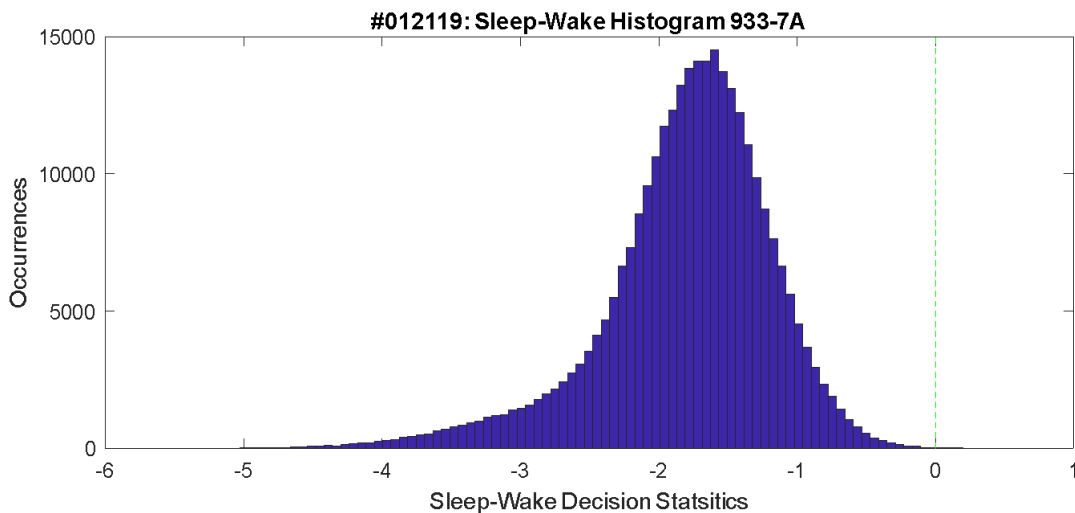


Figure A8. Another example of a signal that appears to be noise-only.

Plotting the time series results in the Fig. A9 below. It looks like there may be some strong wake signals near the light and dark phase transitions.

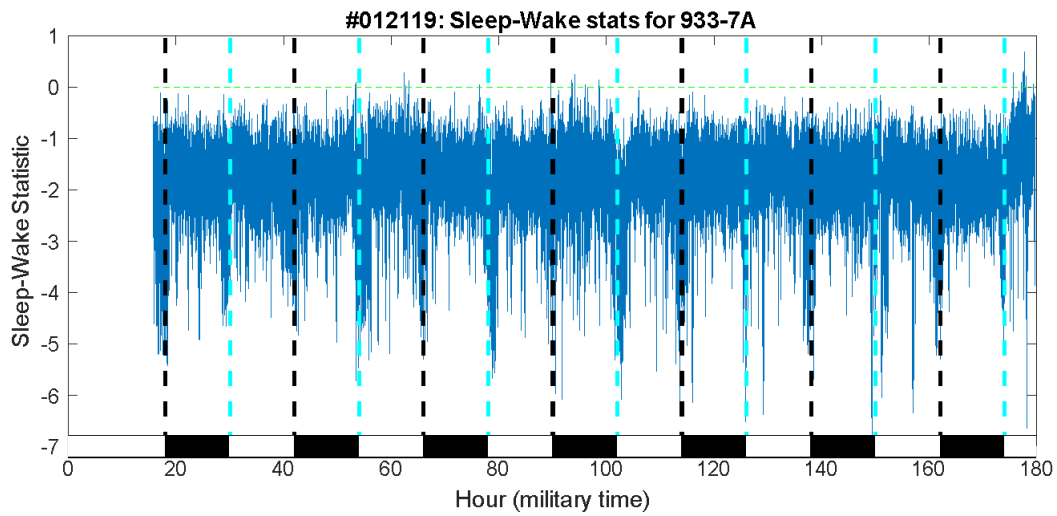


Figure A9. Time series plot of decision statistics that suggests strong wake signals.

Zooming in confirms this Fig A10:

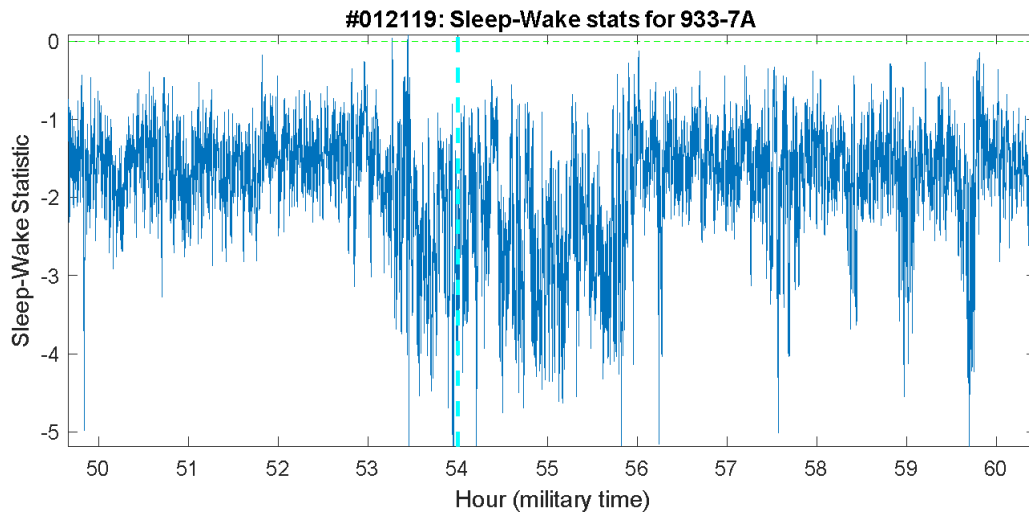


Figure A10. Zoomed in time series which confirms strong wake signals near the transition.

Overlay the pressure signal and observe typical wake and sleep transition patterns based on signal envelope/amplitude characteristics (Fig. A11):

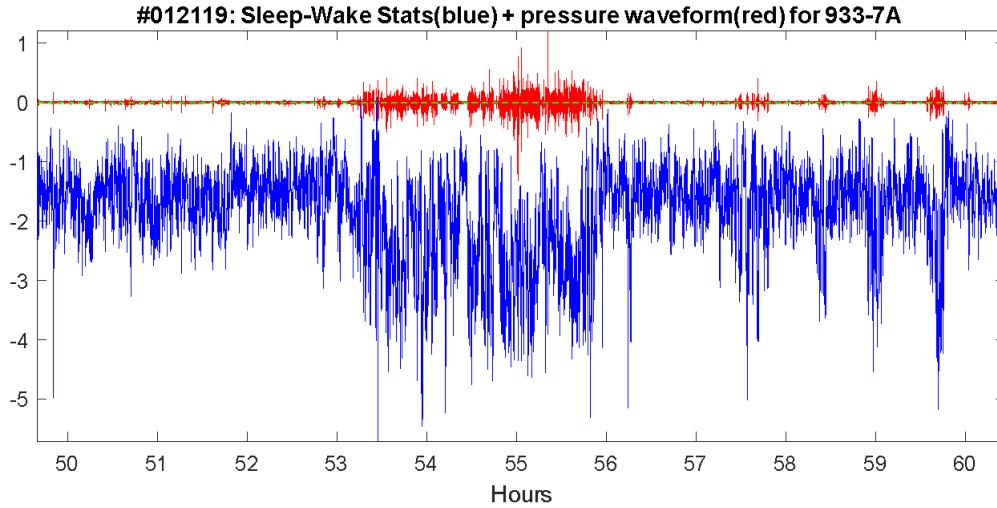


Figure A11. Pressure signal overlay for the plot in Fig A10

When zoomed in further, it appears that 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 the time series data. If zoomed in further, it can be observed that the weak signal during sleep was corrupted by the noise floor. Therefore, a sleep decision was not strongly registered with a higher valued decision statistic.

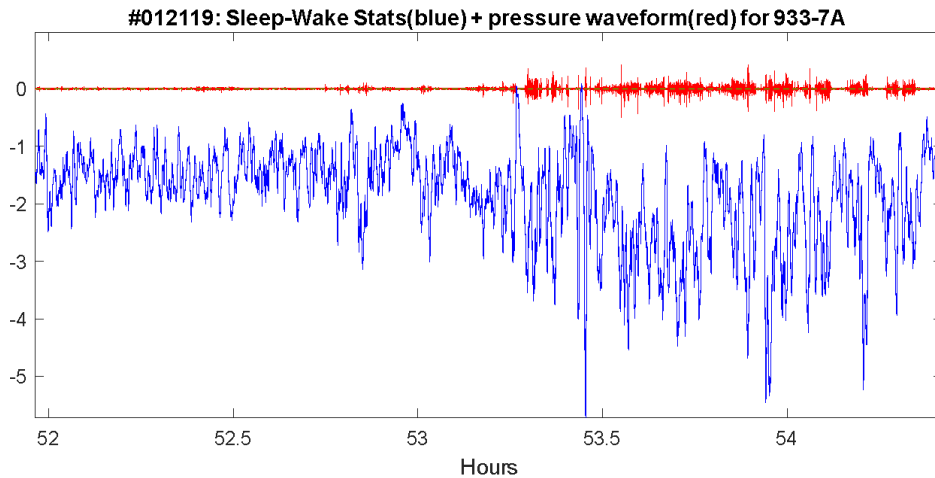


Figure A12. Zoomed in figure to analyze where the decision threshold should be.

After changing the decision threshold, can replot the figure to confirm the threshold has moved and inspect how it affected the results (Fig. A13).

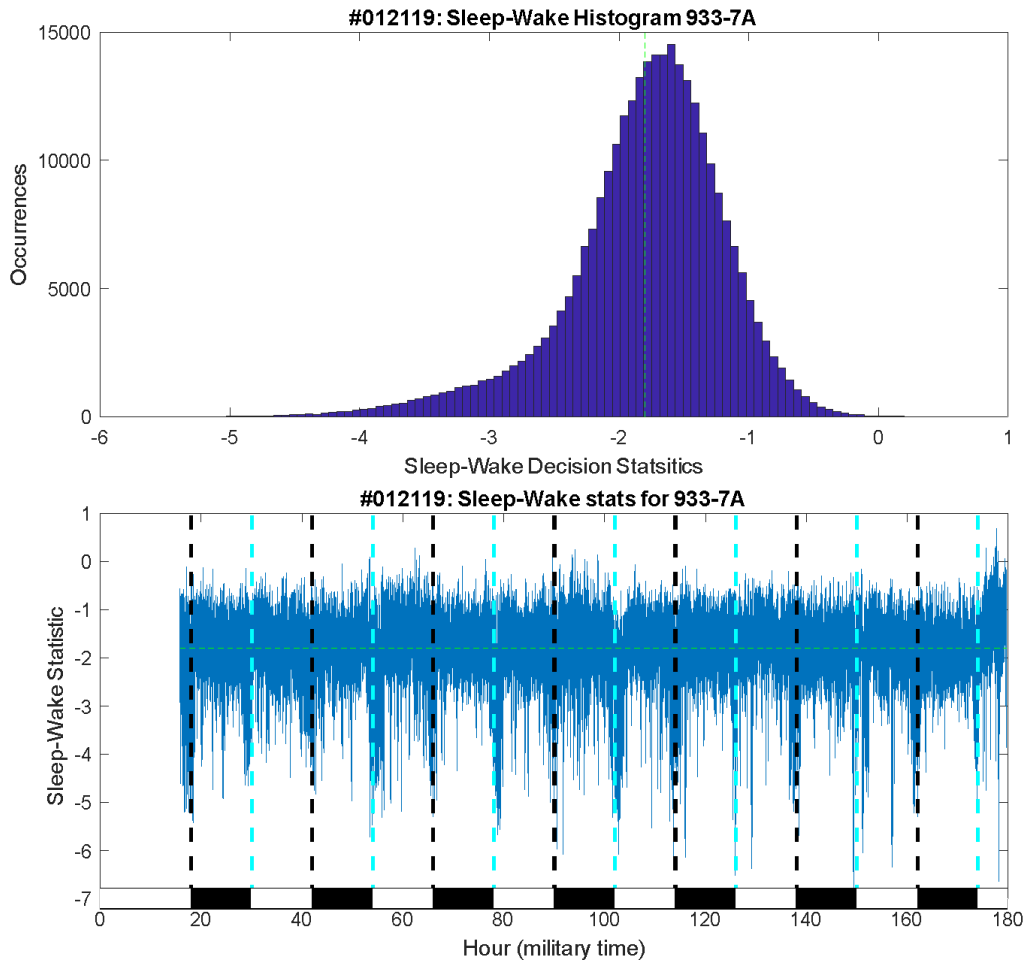


Figure A13. Decision statistics in a histogram and time series to test the new threshold.

Another example of an outlier can be seen in Fig. A14. In this case the sleep percent 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 is typically asleep. This created a lower mode in the sleep range (it disturbed the usual regularity associated with sleep) and confused the automatic threshold setting. The histogram and time series in Fig. A14 show the original threshold location:

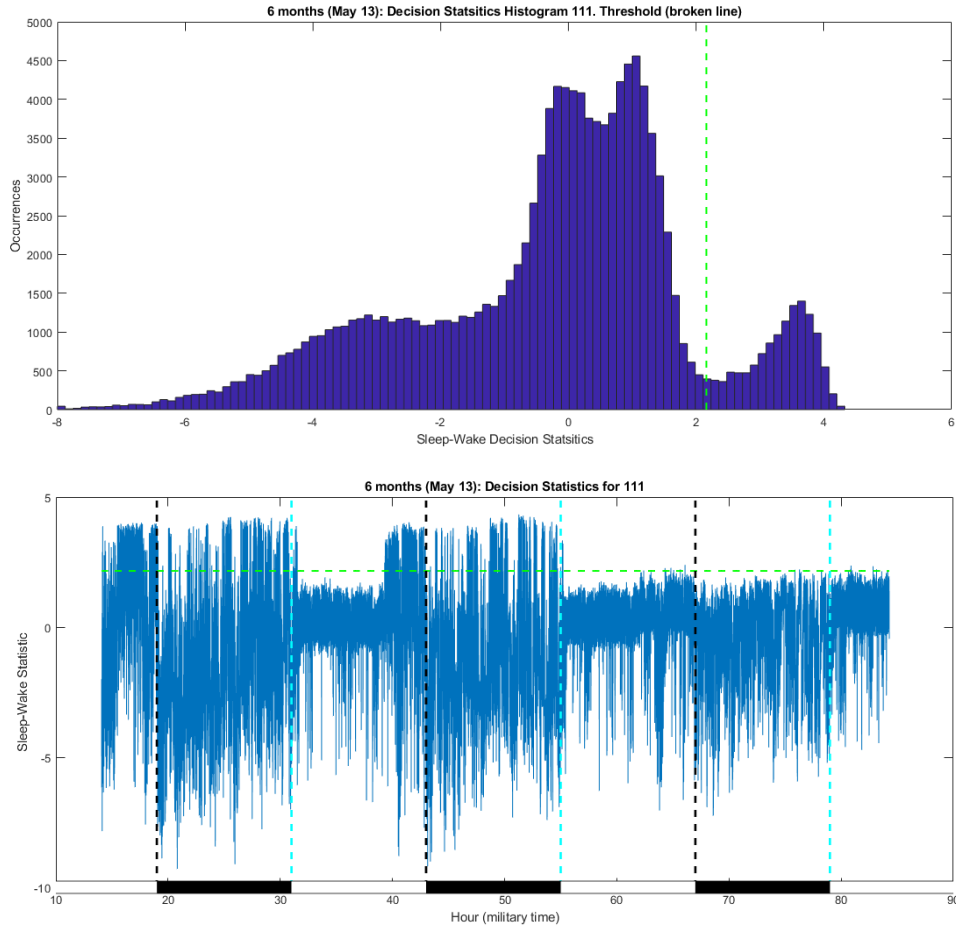


Figure A14. Histogram and time series plots of a segment subjected to regular machine noise.

Observe during the first 2 dark phases, the typically high-valued sleep decision statistics (the noise was not present during this time). This is responsible for the smaller higher mode (around 4 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 plotting the pressure sensor data (Fig. A15) better shows the regular machine noise during sleep bouts (3 minutes on and 3 minutes off) that is corrupting the sleep signal and making it look more like rest rather than sleep.

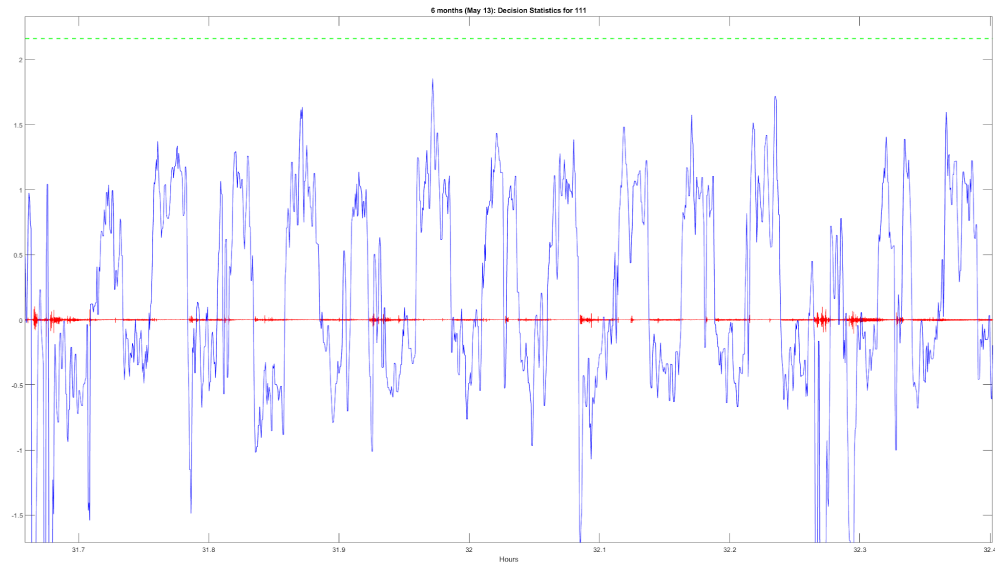
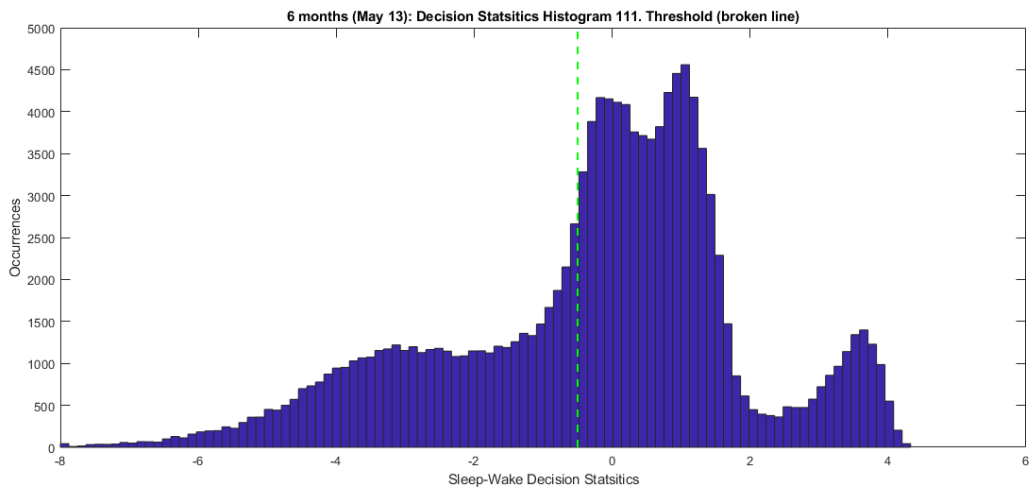


Figure A15. Decision statistic zoomed in with pressure signal overlaid.

As we can see, the threshold line is clearly too high (improperly influenced by the disparity of sleep during the dark phase and light phase 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 2 peaks in the histogram (around 0 and 1). Here it would be recommended to move the threshold to slightly below the statistical values driven down by the noise (there are some short actual activity bouts that look different and are higher than the machine noise, the threshold should exclude these). Moving the threshold too low, would result in some of the wake, or periods of restlessness we see towards the right of this frame, to be scored as sleep. In this case, a threshold of about -0.5 is recommended to try to catch all the sleep but not overcompensate to include the borderline rest-sleep states. Below you can see the adjusted threshold in the histogram and the time series (Fig. A16).



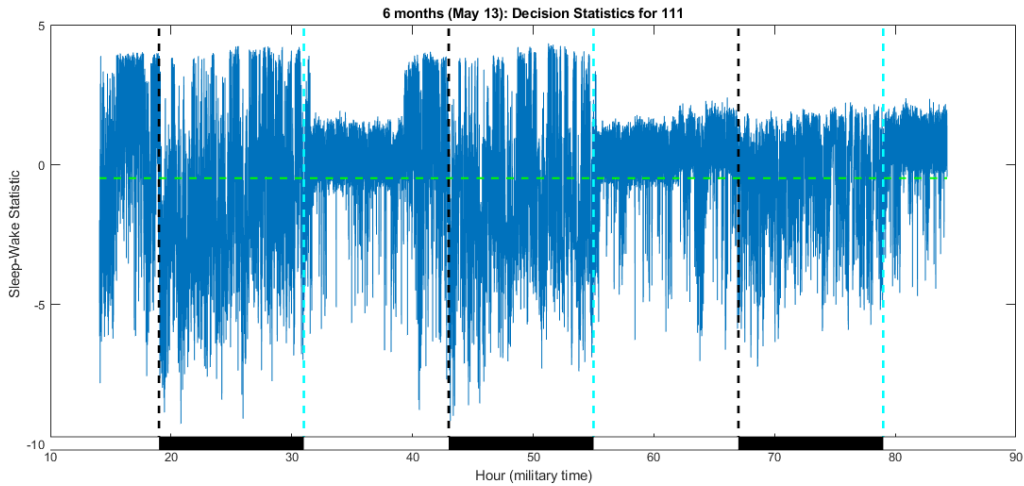


Figure A16. Histogram and time series decision statistics after threshold adjustment.

From the histogram, we see 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 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.