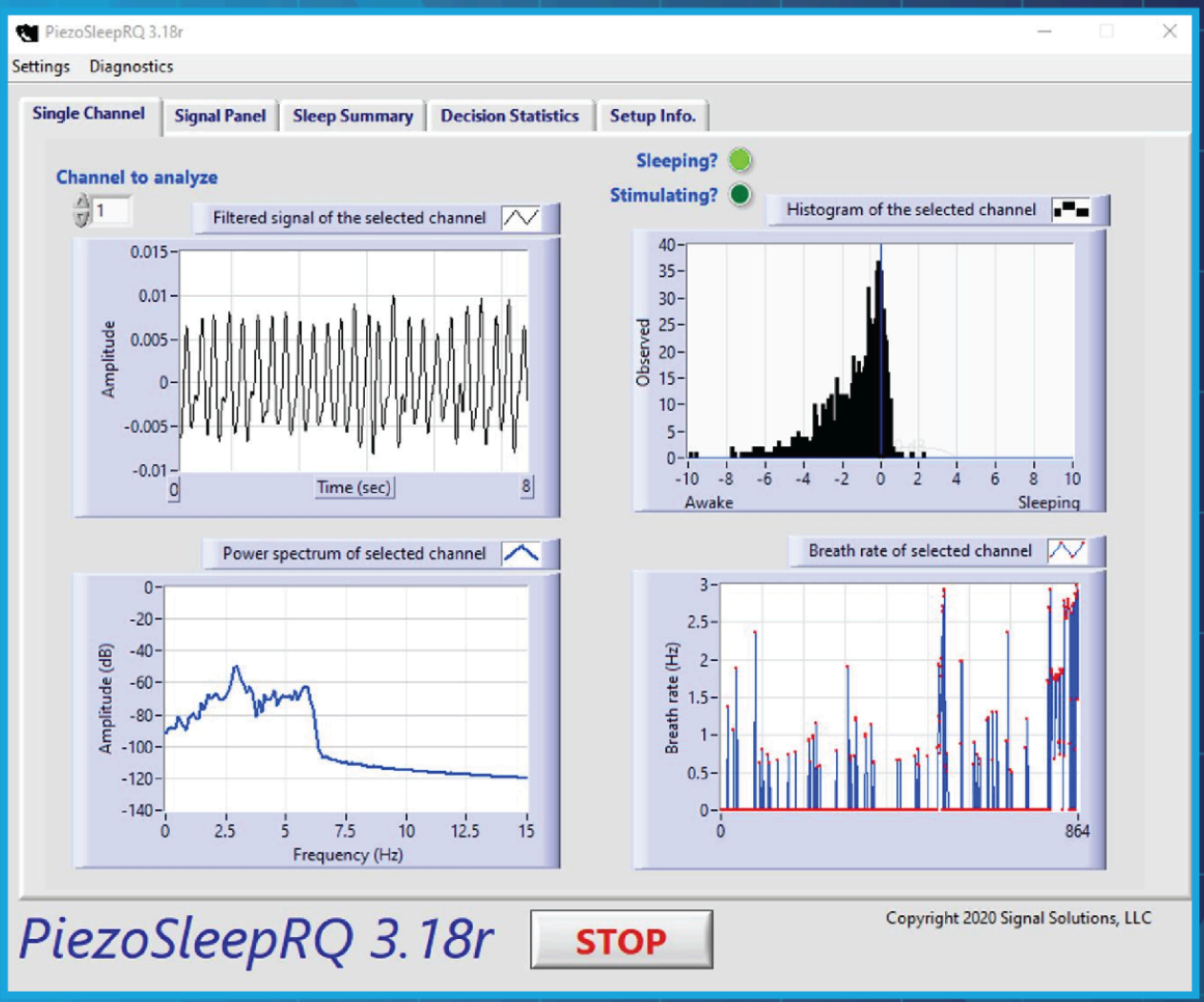
SIGNALSOLUTIONS

**Sensor Systems, Software, Sleep**




# PiezoSleep RatQwake

## USER MANUAL

January 2026

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*January 2026*

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## Contents

# 1. Introduction

The *PiezoSleep RatQwake* software was written and compiled in LabVIEW (National Instruments, Austin, TX) to monitor and record high-throughput animal behavior tracking and apply experimental intervention via non-invasive stimulation.

This program creates several files for classifying behavioral states. The user provides the base name for the recorded data files during the software startup sequence, to which various extensions and suffixes are added to distinguish the different files. The pressure signal from the piezoelectric sensors is sampled at 120 Hz and saved with a ***.bin*** extension. Features associated with sleep and wake behaviors are extracted from the pressure signal and saved every 2 seconds in a file with the ***.FeatVec*** extension. Preliminary estimates of sleep and wake are made in real-time for the purpose of monitoring the experiment and flagging potential problems through extreme numbers of sleep and wake. A more accurate analysis is performed when the ***FeatVec*** file (feature vector) is imported into our analysis program, *SleepStats* (which performs calibration operations if more than 24 hours of data were collected).

A sleep-wake classification is made every 2 seconds. If a channel is estimated to be in sleep, the breath rate is estimated and stored in a file with a suffix and extension ***br.Feat***. When stimulation is applied via the RatQwake, the time of stimulation, as well as stimulation frequency/amplitude/duration for each cag, is saved to a file with the ***_Stim.csv*** extension. Finally, upon completing the software startup sequence, an additional file with the ***_config.csv*** extension is created, which stores the settings used for the experiment (Device, Number of Channels, Stimulation Settings, etc). If the same experimental setup is desired to be used (such as when restarting a recording after cage cleaning), the ***_config.csv*** file can be loaded into the program and edited where needed. While the recording is in progress, the graphical user interface of *PiezoSleep* provides a variety of graphs, statistical summaries, and current sleep-wake states to monitor the data collection for real-time monitoring and control of RatQwake stimulation.

The *PiezoSleep RatQwake* software currently supports monitoring and closed-loop perturbation in up to 8 cages using a Calamari RatQwake Edition acquisition system. The acquisition unit contains a data acquisition (DAQ) module as well as power distribution circuits for the sensor amplifiers and waveform amplification to actuate the RatQwake cages.

The software described in this document automatically detects all devices configured on your PC. Software will run on a PC with Windows 7 operating systems or higher (32 or 64 bit) and a USB port. The installation of the *PiezoSleep RatQwake* software automatically installs drivers from National Instruments to configure the computer to stream data through the USB port, if they are not already installed.

## *A. System Requirements*

Windows 7 later with 2GB or higher RAM, a local hard drive, and at least 1 USB port. A 500GB hard drive or larger and additional USB ports are recommended to facilitate data handling and transfers.

## *B. Installation*

1. To install the real-time sleep-wake monitoring software and supporting National Instruments (NI) software, download and unzip the installation package via the instructions given with your purchase.

2. Transfer the file to the hard drive of the computer where data will be collected and unzip the file. Once unzipped, the directory ***PiezoSleep RatQwake…Installer*** should have been created. Inside this directory, locate the folder labeled ***Volume,*** which has an application file named ***setup***. Double-click this file to start the installation process. Follow the instructions in the prompts to complete the installation.

3. Once the installation process is complete, a shortcut named ***PiezoSleep RatQwake*** will be created on the desktop. Double-clicking on the shortcut will launch the software program. The *PiezoSleep* program can also be accessed from the ***Start*** menu, under the *PiezoSleep RatQwake* folder, or from the hard drive location where it was saved during installation. The software requires the NI LabVIEW Run-Time Engine, which is installed along with *PiezoSleep RatQwake* and is present in the *National Instruments* folder under the ***Program Files (x86)*** folder. When removing this software, the *PiezoSleep RatQwake* program can be identified in the *Programs* option under the ***Control Panel*** from the PC menus and removed.

4. After the software installation is complete, plug the data acquisition box into the PC USB port (and power it up if it requires external power). If this is the first time the device has been connected to the computer, the computer will start installing the required drivers, which may take several minutes. The blue ***Ready*** LED on the device will constantly illuminate when the device is recognized by the computer and is ready for use.

## 2. Computer settings to change before running PiezoSleep

1. Go to the ***Start*** menu and type '*Device Manager*' in the search bar. You will see it listed under the ***Control Panel***.

2. In the *Device Manager*, click and expand the ***Universal Serial Bus controllers*** option to see a list of all USB ports available in the device. To reduce power consumption, the computer will turn off power to USB ports listed as ***Generic USB Hub*** and ***USB Root Hub***. If this happens, the recording will stop, and the program will crash. Therefore, it is important to make sure these settings are such that the computer cannot power down the USB ports.

3. Right-click on one of these USB ports and select ***Properties***. Go to the ***Power Management*** tab and **UN-CHECK** the ***Allow the computer to turn off this device to save power*** option, and press ***OK***.

4. Repeat step 3 for EACH of the USB ports listed as ***Generic USB Hub*** and ***USB Root Hub***. Some may not have a ***Power Management*** tab, so there is nothing to change for these. However, be sure to check all listed USB ports to ensure the one used by the data collection program will not shut down during the experiment.

5. Close the *Device Manager* once you have finished step 3 for all USB ports.

6. Now, ensure the computer will not *hibernate* or go to *sleep* during the experiment when there is no interaction with the computer for an extended period. Return to the ***Start*** menu and type *Power Options*. It can also be accessed from the ***Control Panel*** under a label like ***System*** or ***System and Security***. There are other ways to access this setting, depending on your Windows operating system version.

7. You will see a list of power plans (e.g., Balanced or High performance) or similar options. Find the options to set ***Put the computer to sleep*** and set it to ***Never***. If you are using a laptop, you will see the options ***plugged in*** and ***on battery***. Change the ***plugged in*** setting to ***Never***. If you don't see an option for never, you can type it in.

8. Click on the ***Change advanced power settings***.

9. Within the ***Sleep*** options, you may see ***Allow hybrid sleep***; if so, set it to ***Never***. For laptops, set the ***plugged in*** option to ***Never***.

10. Repeat step 9 for the ***Hibernate after*** option under ***Sleep***. Remember that you can type in the word *Never* in these options.

11. Collapse (come out) the *Sleep* option and go to the ***USB settings*** option. Expand the ***USB selective suspend setting*** and set it to ***Disabled***. For laptops, disable the ***plugged-in*** options within the ***USB settings***.

12. Go back to the ***Start*** menu and type *Windows Update*. It can also be accessed from the ***Control Panel*** under ***System and Security***.

13. Under *Windows Update*, click on the ***Change settings*** option located on the left column of the window.

14. Click on the pull-down menu under ***Important updates*** and select the ***Never check for updates (not recommended)*** option. This will prevent the computer from restarting in the middle of a recording. It is recommended that you manually update Windows from time-to-time. Manual updates can be done by clicking on the ***Install updates*** option located under *Windows Update*.

# 3. Data Acquisition and Monitoring

## *A. Starting a Data Acquisition Session*

Launch *PiezoSleep_RatQwake* either from the desktop shortcut, from the start menu, or from the location it was saved/installed on the hard drive. Afterwards, the windows shown in Fig. 1 should appear.

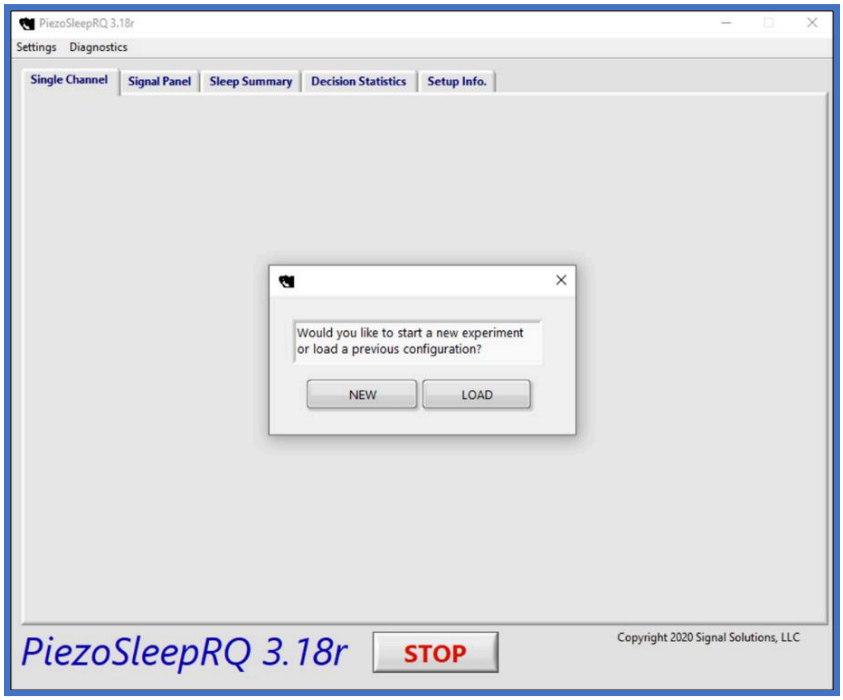



**Figure 1:** Starting windows for the *PiezoSleepRQ* program. From the dialog window, select whether you would like to start a new experiment or load a previous experiment configuration.

Select either to start ***NEW*** experiment or to ***LOAD*** a previous experiment's configuration via a **_config.csv** file.

- If ***NEW*** is selected, a series of prompts to select Device, Channels, Stimulation Settings, etc. will be displayed for the user to configure the experiment.
- If ***LOAD*** is selected, a dialog to select a ***_config.csv*** file will appear. Once a file is selected, the file will be loaded, and a prompt will appear to specify the new data file. The settings will then be displayed to the user, allowing for editing if needed (see ***File Section*** for further information related to the configuration file).

If ***NEW*** is selected, a window will appear to select the acquisition device and specify the number of recording channels. If multiple DAQs are connected to the computer (from other National Instruments systems), after clicking on select, a drop-down menu will appear listing all the connected DAQs as shown in Figure 2 (left). In most cases, you will see only one device and select that one. If there are multiple devices associated with other data collection activities, then select the one NOT associated with another system (i.e, the newest device plugged into the system), then press ***OK***.

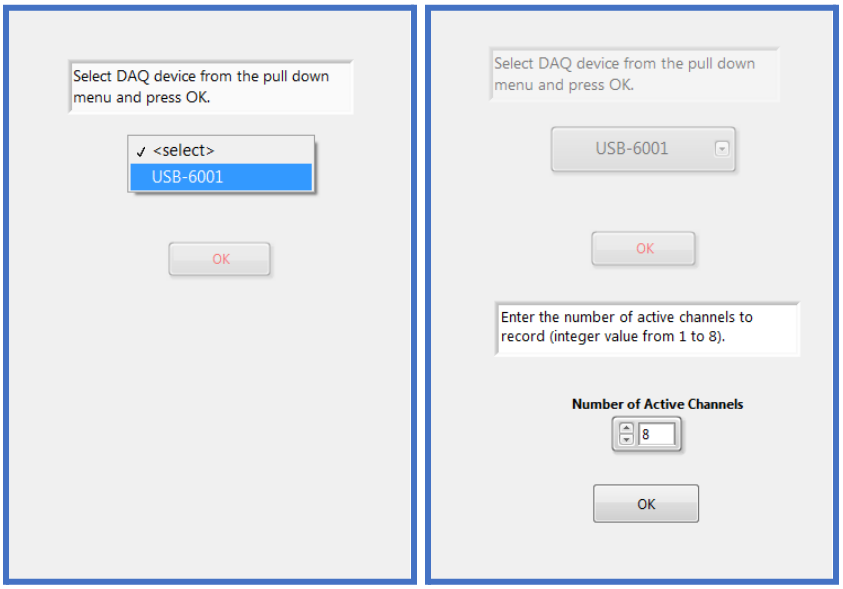



**Figure 2**: (Left) Prompt to select a DAQ. A listing of all DAQs currently plugged into your PC will be stored in a dropdown menu. Click on a name inside the list and press OK. (Right) Once selected, a box will appear to enter the number of recording channels.

After configuring the device and channel selection, another dialog box will appear, as shown in Figure 3. The dark-to-light and light-to-dark transition times can be entered in the designated area, where the times are in military format (0 to 24). These time values are stored in the data file headers and used in a later analysis program to compare sleep behavior during the light and dark periods. If light and dark onset times are not critical, then these can be left in their default settings and disregarded or changed later in the *SleepStats* analysis program. This entry will not affect the data acquisition. In this same window, the animal IDs can also be changed to meaningful labels if desired. These ID labels are also stored in the header so subsequent

analysis programs can identify each channel with these labels. IDs can also be imported from an ***.xlsx*** file using the button to the left of the ***OK*** button. The Excel file must contain the IDs in the first column of the *.xlsx* file with no header row (cell A1 should be the label for animal in cage 1). When finished entering this information, click ***OK*** to proceed.

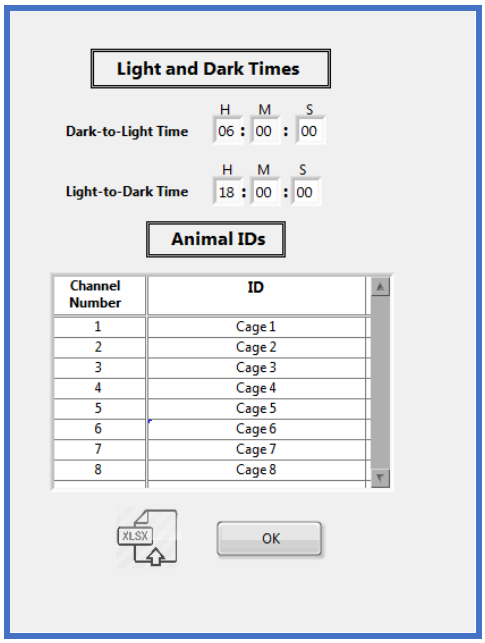



**Figure 3:** Prompt to enter labels for recorded channels and the light and dark transition times used during the experiment.

The program then prompts for a file name, as shown in Figure 4. Animal motion/pressure data will be saved to this file for later processing or checking the signal quality of the recording. It is recommended to give this file a descriptive name that identifies the nature of the experiment or mice and the date the experiment began. It is also recommended that NO extension be given to the file name, since *PiezoSleep* adds its own extensions that are used in later programs (the *bin* extension is given to files with the raw sensor data that are useful for reprocessing or updating data when new algorithms are released). The software, *SleepStats*, which analyzes data after the experiment, uses the base name to look for processed files and opens them automatically. If they have different names and extensions, the automatic loading will not work, and you will be prompted to search for them with a file/directory navigator.

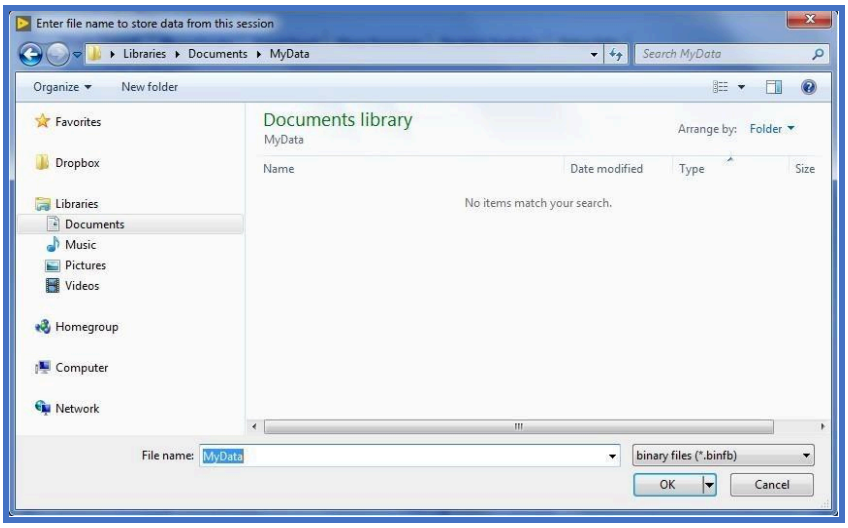



**Figure 4:** Prompt to enter a filename where data is to be stored during the experiment

After selecting the file name to store data, a window will appear to configure the RatQwake stimulation settings (Fig. 5). If stimulation is not desired, this window can be bypassed by pressing ***OK***. If stimulation IS desired:

a. Enable Cages for Stimulation:

For cages to be enabled for stimulation, check their boxes in the upper left-hand corner, ***Cages to Stimulate***.

b. Select which Cages to Stimulate with External Trigger input signal (if available):

If the Calamari RatQwake hardware unit is equipped with coax connections for TTL inputs and outputs, then the hardware supports the External Trigger option. The small LEDs near these connections, labeled ***In*** and ***Out***, will illuminate when the TTL signal is active on the corresponding port. If the ***Ext. Trig*** checkbox is enabled, the cage will be stimulated upon the corresponding TTL input transitioning to a HIGH state, rather than according to the Stimulation Protocol (e.g., open-loop, sleep-based, etc). Stimulation will persist until the TTL input signal returns to a LOW state. TTL outputs are also provided, which transition to a HIGH state anytime stimulation is occurring, regardless of whether

it was applied via the Stimulation Protocol or the External Trigger. (Note: The 'in' LEDs will illuminate with a TTL HIGH state, even if the cage is not enabled for External Trigger).

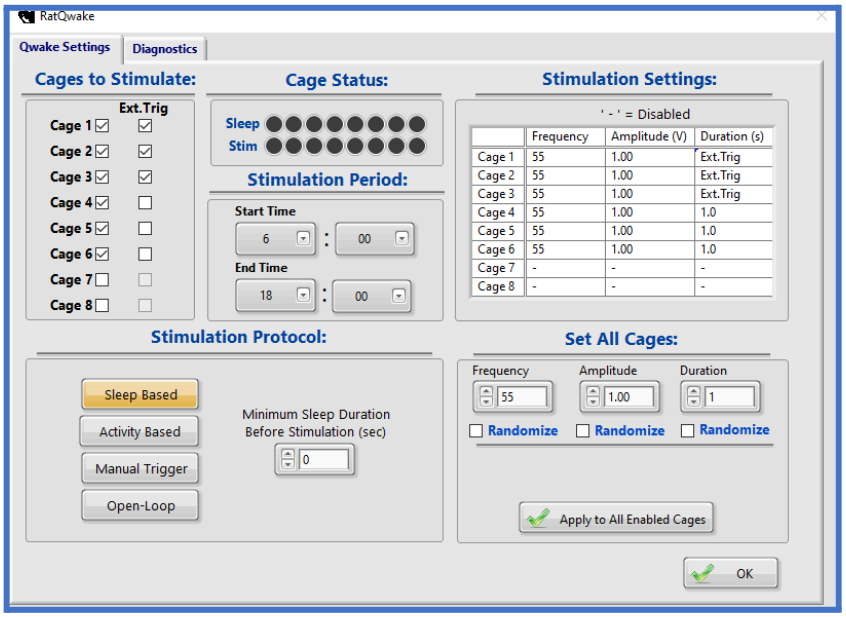



**Figure 5:** Select Stimulation Settings. Depending on the selected mode, additional inputs may appear. Enter the desired settings and press OK. These values can be reconfigured in real-time once the software is running. To access this window again, click on *Settings->Qwake Settings* in the menu bar at the top of the main program.

c. Configure Stimulation Settings:

Once a cage is enabled, the corresponding row in the Stimulation Settings table will be populated as ***Not Set.*** Stimulation parameters (frequency, amplitude, duration) can be individually specified by typing the corresponding entries into the Stimulation Settings table. Values will be coerced to their closest allowable value if entries outside the acceptable range are entered. A given cage's frequency, amplitude, or duration will be randomized if the corresponding index in the table is set to *Random*. If the same settings are desired to be applied to all active cages, the controls below the table can be used. Upon clicking the ***Apply to All Enabled Cages*** button, the values in the Frequency, Amplitude, and Duration boxes will overwrite the table values for all enabled cages. If a parameter (e.g., frequency) is desired to be randomized, the corresponding ***Randomize*** check box can be checked before clicking the Apply button. Once any table values are set

to *Random*, additional controls will appear for specifying the range within which to randomize. The apply button will disappear once pressed, and will reappear if a cage is enabled/disabled or if the user clicks into one of the Frequency, Amplitude, or Duration controls. Stimulation Table values of 0 are not allowed. If table values of ***Not Set*** or ***0*** are present when completing the configuration (by clicking ***OK***), a dialog will appear informing the user to confirm that the settings are valid.

d. Stimulation Period:

Active stimulation periods over the 24-hour day can be limited to a particular time period using the controls in the ***Activation Period*** section. If stimulation is only desired during the light-period, for example, light onset and offset times can be set as the Activation Start and End times, respectively. Setting Start Time as 0 and End Time as 24 will allow stimulation anytime the Stimulation Protocol-dictated criteria are met.
**NOTE:** End Time must be after Start Time. Once the desired stimulation configuration has been specified, press the ***OK*** button to write the settings to the Calamari's internal memory to be used during the experiment. These settings can be modified during experiments, if needed (See section 3-G). Settings will be written to the ***_config.csv*** file for reference, or for loading as a template for future experiments.

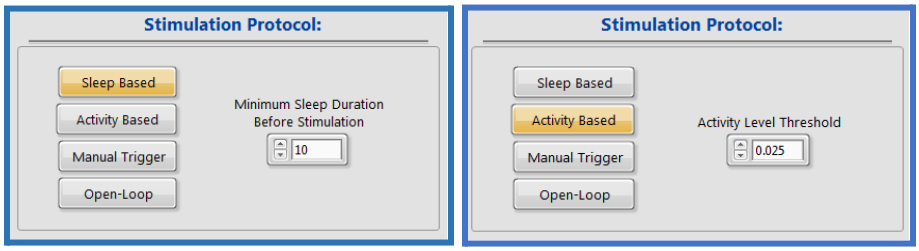
e. Stimulation Protocol:

Apart from being externally triggered via TTL, stimulation can be applied according to four protocols: *Sleep Based*, *Activity Based*, *Manual Trigger* (i.e., On Demand), and *Open-Loop* (Fixed or Random interval). The protocol dictates *when* stimulation is applied. Under any protocol, when stimulation is applied, it will be applied to all enabled cages with settings specified in the ***Stimulation Settings*** table.

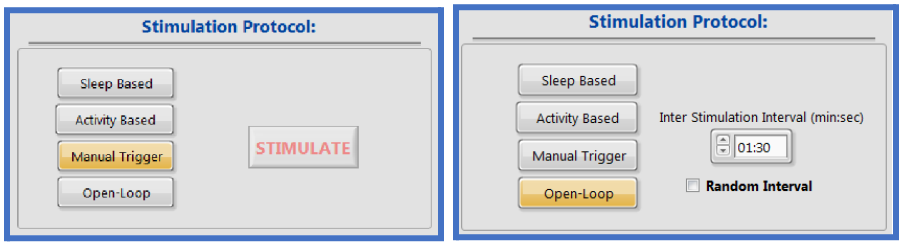
- If ***Sleep Based*** is selected, stimulation will be applied to any cage that is detected to have been in sleep for a minimum amount of time, which the user can specify in the *Minimum Sleep Duration Before Stimulation* field (Note: This value is specified in

seconds). A value of 0 will apply stimulation of enabled cages immediately upon detection of sleep.

- If ***Activity Based*** is selected, the simulation occurs when the Activity level falls below a user-specified Activity Level Threshold. *Activity Based* protocol allows the user to 'tune' when stimulation is applied. This option is useful if the *Sleep Simulation* is allowing too much rest or transitional sleep to occur, or if stimulation of quiescent wake behavior is desired.




- If ***Manual Trigger*** is selected, a ***STIMULATE*** button will appear, which, when pressed, will trigger a single stimulation to enable cages with settings specified in the Stimulation Settings table. The *STIMULATE* button is only functional from the RatQwake tab of the software after the software startup sequence has been completed.
- If ***Open Loop*** is selected, an input will appear to specify the interval (in minutes and seconds) at which stimulations will occur. In Open-Loop, all enabled cages will be simulated simultaneously, independent of their sleep-wake state or activity level. If the 'Random Interval' box is checked, stimulation intervals will be randomly assigned between 0 and 60 minutes.




7. After selecting the stimulation configuration, the program displays your selections as shown in Figure 6. The display includes the DAQ device, number of active channels, file path, light and dark onset times, animal IDs, and RatQwake configuration. It also contains a re-selection menu,

which allows you to re-select the DAQ device, animal ID names, stimulation mode, or filename and location. If no re-selection is needed, then select the last option and click *OK* to continue. Upon pressing OK, a ***_config.csv*** file containing these settings will be automatically generated and will be stored in the data file path. It is recommended that the location of the stored files be noted for later retrieval and archiving.

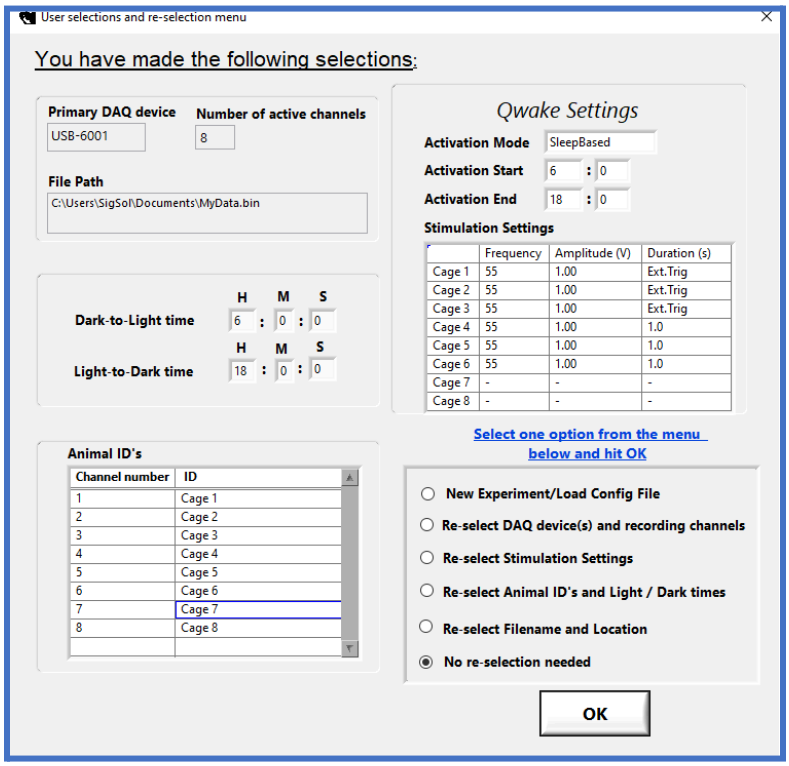



**Figure 6:** Prompt to check entries made and modify if incorrect information was entered before the data collection process begins.

8. The program now starts recording and displaying data. Under each tab, the waveforms/ statistics will begin to appear in a few seconds. Details of these tabs are explained in the following paragraphs.

9. When data collection is finished, click the ***STOP*** button located at the bottom of the window. A new test can then be started by clicking the arrow button, located on the top left corner, or the program can be terminated by clicking the ***X*** in the upper right corner to close the window.

## *B. Selecting and Monitoring a channel signal (Single Channel tab)*

The ***Single Channel*** tab displays the most recent 8 seconds of the recorded signal along with additional graphical descriptions. As shown in Fig. 7, the tab consists of four panels: the pressure signal (top left), the sleep decision statistic (top right), the power spectrum (bottom left), and the breath rate frequency (bottom right) for a given channel. To facilitate real-time observation, the *PiezoSleep* program computes and updates the power spectrum, breath rate, and the sleep decision statistics every 2 seconds. The power spectrum shows the signal's power distribution over a range of frequencies. The power spectrum example in Fig. 7 shows one dominant peak at around 3 Hz. The amplitude on the y-axis is expressed in the logarithmic unit decibels (dB).

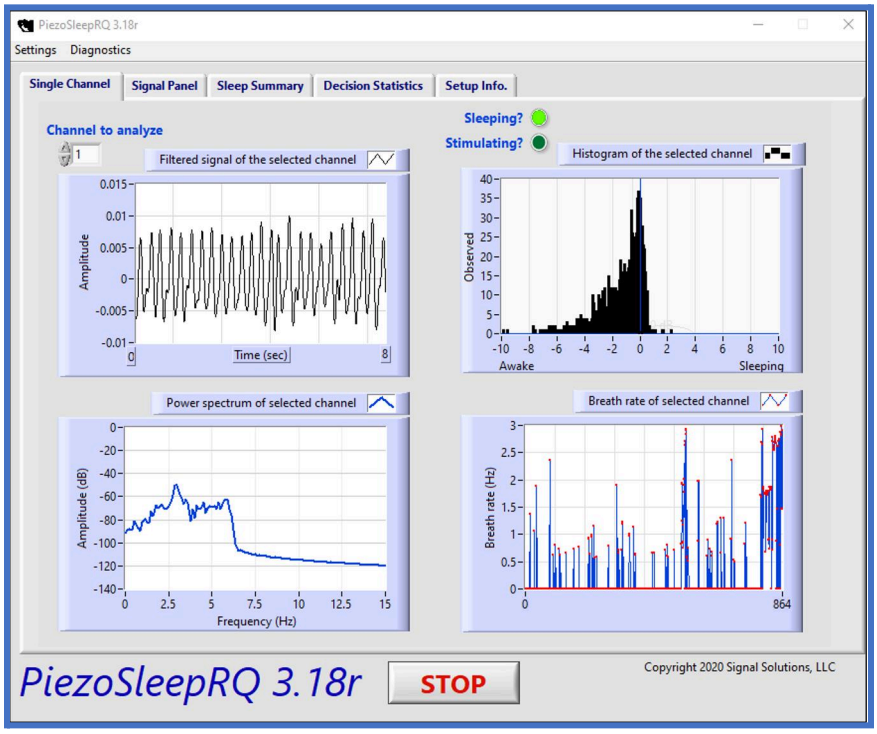



**Figure 7:** An example of software acquiring data and classifying the signal as sleep or wake.

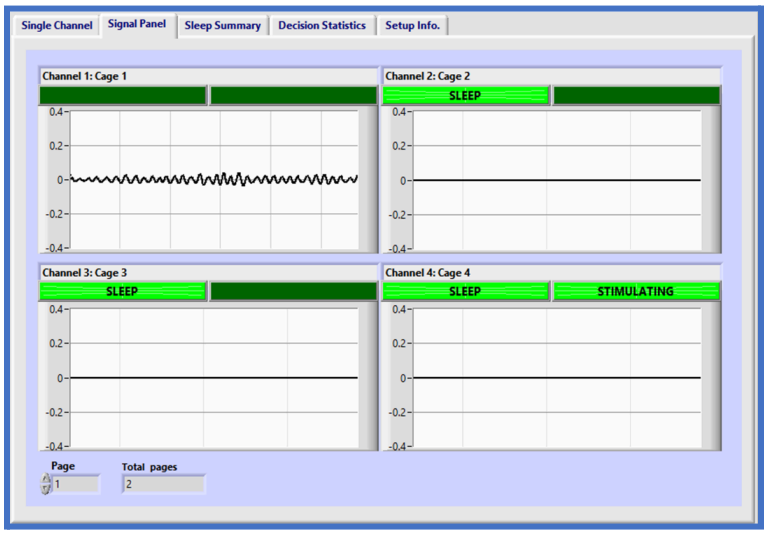
The sleep decision statistic comprises a histogram plot showing the sleep-wake distribution for the selected channel. The sleep-wake state (with about a 4-second lag from real time) is shown via a green LED, located just above the panel, which lights up to indicate sleep. Detection is based on comparing the decision statistics to a sleep threshold, which by default is zero and is

depicted on the histogram by a blue vertical line. Larger positive decision statistics indicate a greater likelihood of sleep, while negative decision statistics indicate a greater likelihood of wake. In Fig. 7, the green LED is lit, indicating a current sleep state, and the histogram shows a larger concentration on the positive side, indicating that the animal has been asleep for some time. **NOTE: The real-time statistics and classification are about 10% less accurate than the decision statistics and thresholding performed in that analysis program. *SleepStats* can calibrate the amplitude-based measurement for better performance. This can only be done after the full recording for at least 24 hours.** Therefore, all assignments of rat sleep behavior should be made using *SleepStats* after the recording is complete. The purpose of real-time monitoring is to detect potential outlying behavior, sick mice, or bad connections while the experiment is running, and potential corrections can be made. The breath rate plot displays the most recent 32 minutes of data, updating itself once every 2 seconds. For the first 32 minutes after starting the program, the breath rate plot will accumulate data, and from there on, plot the most recent 32 minutes of data. The breath rate plot in Fig. 7 shows that the breathing frequency of the animal was around 3 Hz.

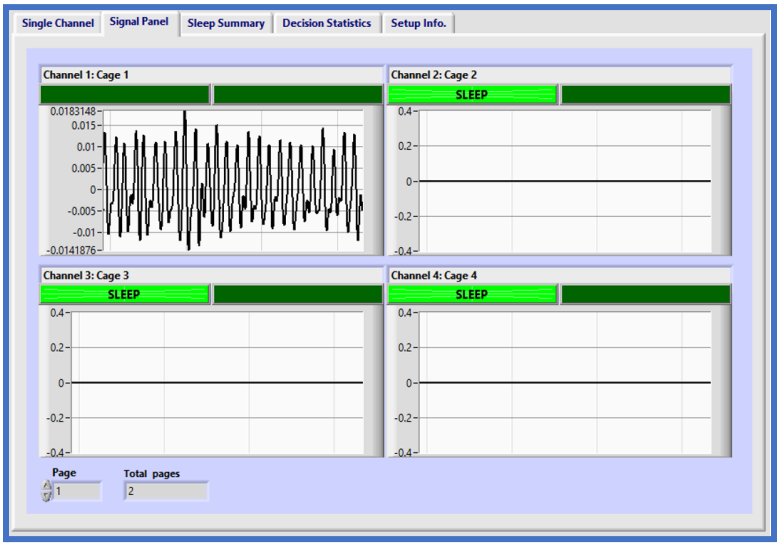
## *C. Monitoring sensor signals for all active channels (Signal Panel tab)*

To view sensor signals for the active channels simultaneously, click the ***Signal Panel*** tab. The *Signal Panel* will appear, as shown in Fig. 9, and display 4 active channel signals over the last 8 seconds. If there are more than 4 active channels, the Page text box can be incremented to page through the rest of the channels. The animal IDs with their corresponding channel number are displayed over each plot. There are two thin green bars (virtual LEDs) above each plot on the *Signal Panel*. The left indicator is the sleep indicator, and will illuminate green and display ***SLEEP***, as seen for channel 1 in the figure. The right indicator will illuminate and show the text ***STIMULATING*** when stimulation is being applied. The y-axes of the graphs on the *Signal Panel* are fixed from -0.4 to 0.4 volts (volts are proportional to pressure). These graphs, by default, do not auto-scale the signal like the filtered signal plot on the Single Channel panel, so relative amplitudes can be observed. The primary purpose of these graphs is to view signals from all

cages quickly to see if there are problems (broken connections, amplifiers not plugged in, dead mice, etc.). However, by right-clicking on the y-axis of these graphs, you can turn on the auto-scale to see more detail for weaker signals. To go to a fixed scale, set to manual scale, double-click on the y-axis maximum and minimum numbers, and type over to set the limits. There is a 2 to 4-second delay between what is happening in the cage and what appears on the screen.




a)




b)

**Figure 8:** *Signal Panel* tab displaying piezoelectric sensor signals for 1 active channel. The *Page* box is used to switch to other active channels when more than 4 channels are active. (A) Y-axis fixed (default), and (B) y-axis switched to auto-scale, shown with a simulated sleep signal.

## *D. Monitoring percent sleep for all active channels (Sleep Summary tab)*

The percentage of sleep for each channel can be observed at any point during the recording in the ***Sleep Summary*** tab shown in Figure 9. The percentage is based on the sleep threshold and the histogram formed from the accumulating data. Outliers can be identified here early in the experiment and can be followed up with direct observation of the animal or checking the function of the sensor (e.g., ensure the cable is plugged in). Again, *SleepStats* will provide more accurate numbers after the experiment has been completed.

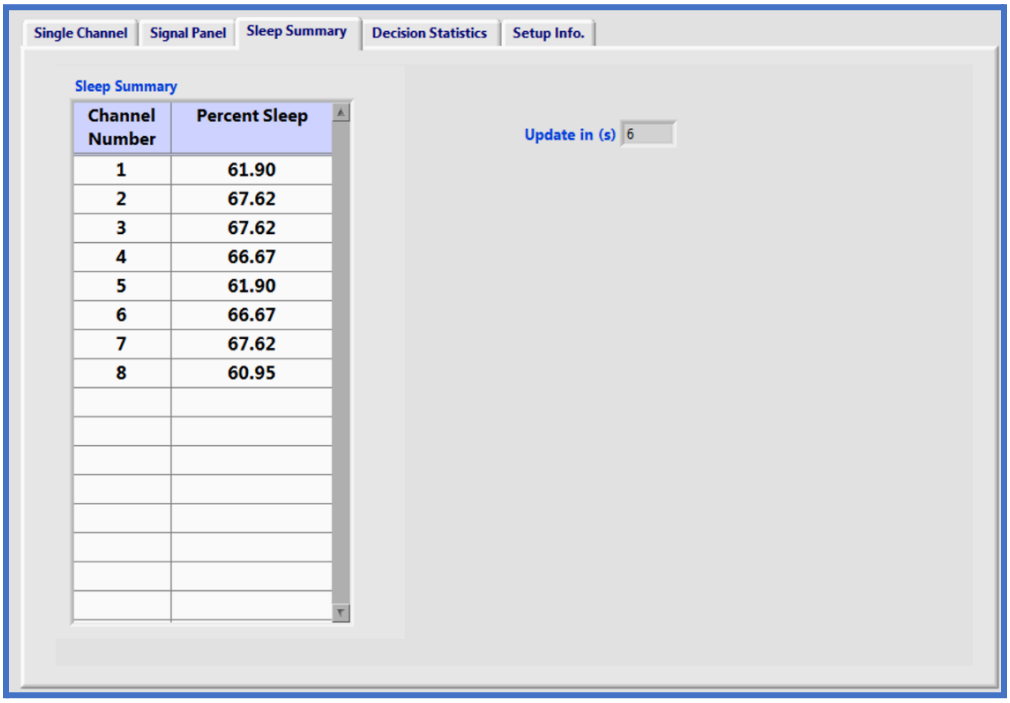



**Figure 9:** The sleep summary tab shows the percent time spent in sleep for each cage.

## *E. Monitoring decision statistics for all active channels (Decision Statistics tab)*

To simultaneously view the sleep-wake decision statistics for the active channels, click the ***Decision Statistics*** tab, which shows histograms for 4 active channels at a time, as illustrated in Figure 10. The animal IDs with their corresponding channel number are displayed over each plot. If there are more than 4 active channels, the ***Page*** text box can be incremented to view

results from the other channels. This provides a quick way to examine the data collection and sleep-wake behavior over the experiment. Unusual behaviors or data collection problems can be identified during the experiment, which can be addressed by direct observation of the animal. For example, after running the program for 24 hours with a **normal/control** animal, a bimodal distribution should appear in the histograms from mapping sleep signal dynamics toward positive values and wake signal dynamics toward negative values. If the histogram is not bimodal, it could be the result of noise or weak signals, for which there are a number of causes:

- an electrically or mechanically noisy environment
- poor animal contact with the piezoelectric sensor on the floor of the cage (sometimes caused by too much bedding or the cage shield not being properly seated)
- faulty amplifier or sensor
- unusual animal behavior not observed in the training phase of the classifier (i.e., animal is ill, dead, or has a very unusual respiratory pattern during sleep)

The bimodal pattern in the histogram below is exploited by adaptive thresholding, used in later analysis programs, which seeks a minimum point between the clusters (modes) of decision statistics. Real-time analysis is limited in that it uses the same threshold over all channels and can vary by as much as 8% from the later analysis using an adaptive threshold. The primary purpose of real-time monitoring in the acquisition program is to alert those monitoring the experiment. If problems arise, they can be verified (by direct observation of the animal) or corrected during the experiment. More accurate sleep and wake parameters are generated with the *Sleepstats* program.

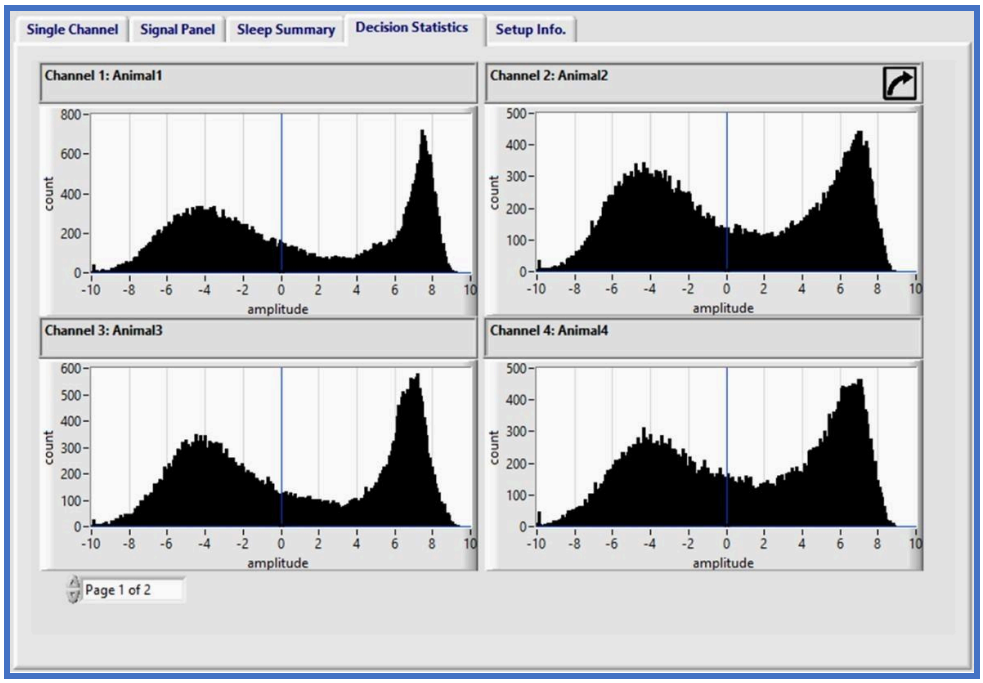



**Figure 10.** The *Decision Statistics* tab shows histograms of 4 channels simultaneously. The *Page* box can be used to switch to other active channels if more than 4 channels are active. The undock arrow in the upper right corner displays the current tab in an independent window, so other panels can be observed simultaneously.

## *F. Current file and setup information display (Setup Info tab)*

After data collection has begun, current information and settings will be displayed on the ***Setup Info*** tab, as shown in Figure 11. The display includes: Start Time, Number of active channels, Primary DAQ selections, File Path, and Light and Dark transition times. These selections were made by the user at the start of the data acquisition session. The start time is stored in the data file header, so it is saved with the data to create an absolute time axis for computing statistics related to the light and dark periods, as well as confirming the date the experiment began. The ***LED Dimming Control*** can be toggled to dim the LED indicators on the front of the *Calamari RatQwake* unit (see Description of Diagnostic LEDs in Hardware and Assembly Manual).

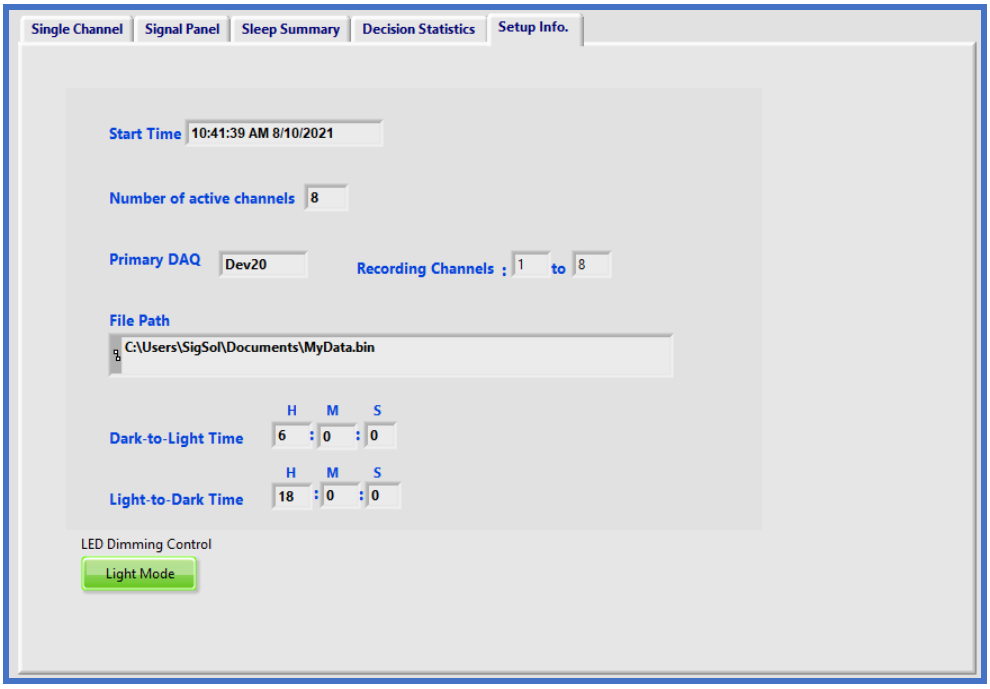



**Figure 11:** Check on setup parameters.

## *G. View and Modify Stimulation Configuration (Settings->Qwake Settings)*

The stimulation configuration and associated diagnostics can be reopened via the menu bar in the main program by selecting *Settings->Qwake Settings* (Fig. 12).

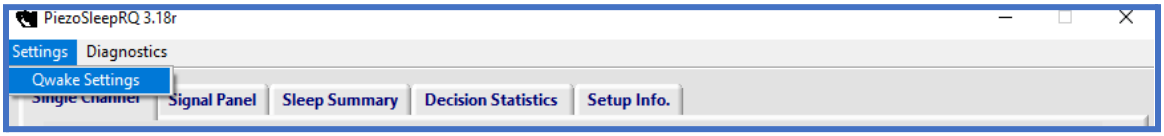



**Figure 12:** Accessing Qwake Settings from the menu bar in the main program.

Once opened (Fig. 13), both the Qwake Settings tab and Qwake Diagnostics tab are accessible. By default, the ***Qwake Settings*** tab is locked to prevent it from being unintentionally modified during an experiment. If the configuration needs to be modified, the panel must first be unlocked using the button in the lower right corner of the panel. If settings are modified, additional buttons will appear, giving the user the option to save or cancel the changes. If these buttons are visible, any changes made have not been saved to the device, and the device will

continue to operate with the previously saved (or initially specified) settings. If the ***Cancel*** button is pressed, the values in the panel will be restored to their last saved values. If changes are saved, the changes are sent to the internal memory of the *Calamari RatQwake* unit for future execution, and the ***_config.csv*** file is updated to reflect the changes. If invalid settings are attempted to be saved, a prompt will be displayed to request the user to verify the configuration. Real-time indication of Sleep detection and stimulation can be seen on the round indicators in the ***Cage Status*** section, which illuminate green when sleeping or stimulating.

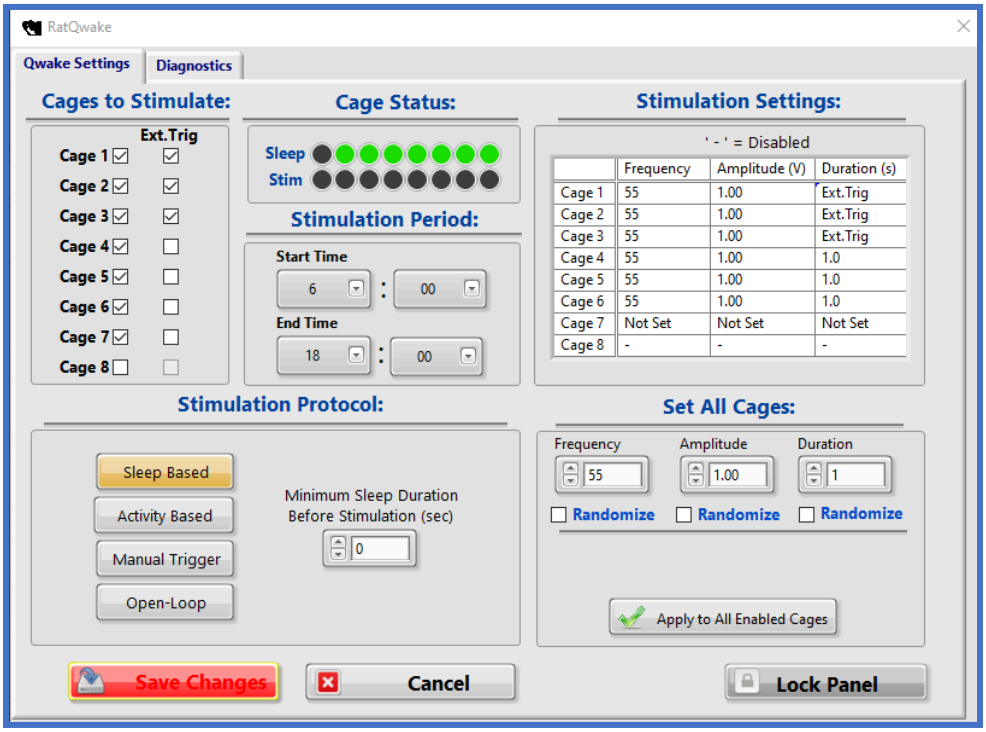



**Figure 13:** Upon modifying the RatQwake panel (here, Cage 7 was enabled during the editing process), buttons to either save or cancel changes will appear. Until settings are saved, the previously saved settings will continue to be used by the device. Pressing the cancel button will restore the previously saved settings to the panel. Pressing the Save Changes button will write the settings to the device memory and update the *_config.csv file*.

## *H. Check System and Troubleshoot (Diagnostics Menu)*

The ***Diagnostics*** tab in Fig. 14 can be accessed from the menu bar in the main program by selecting *Diagnostics->Qwake Diagnostics*. The *Diagnostics* tab can be used to check various aspects of the system, including: power supply voltage, proper connection to RatQwake shaker transducers, and system temperature. Issues related to power and temperature are detected automatically and will cause the software to display a warning. If 12V power is not detected, the On/Off switch will flash red and display the text ***12V not present***. If the voltage of the power supply is detected to be outside the allowable range, the 12V plug icon will flash red. If the system is detected to be in an 'overheating' condition, a flashing indicator that reads ***High Temperature*** will appear above the back-panel image. Upon pressing the ***Run Diagnostics*** button, the Software will check the status of the shaker output connections and change the color of the black rectangle corresponding to that channel's output on the software back-panel image. The legend below the image can be referenced to determine what status is reported.

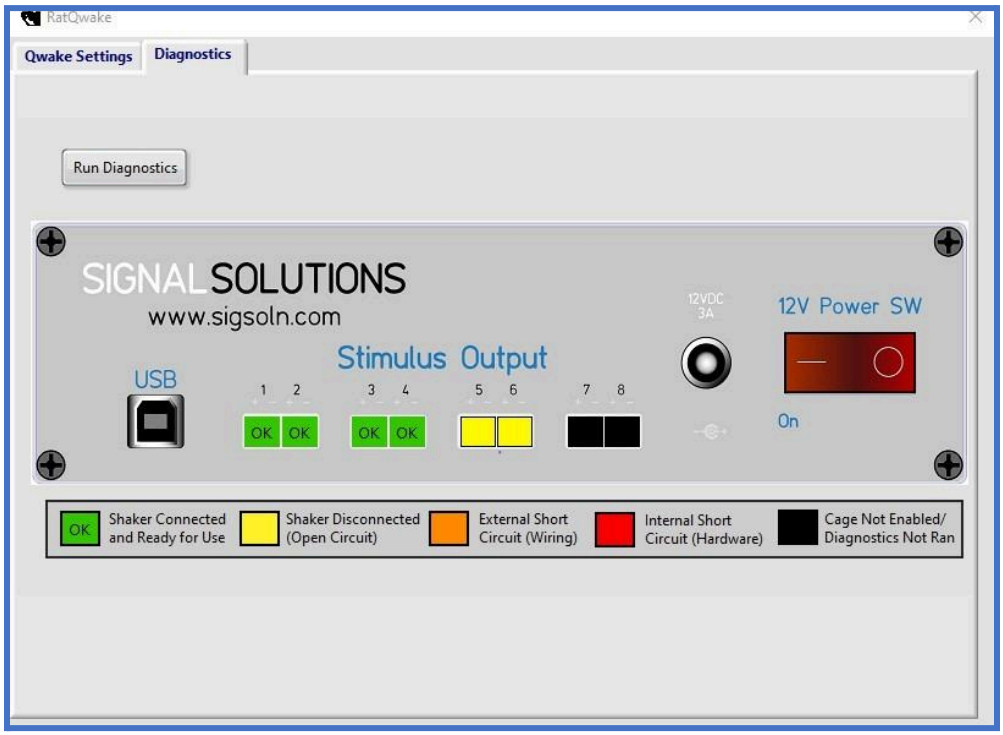



**Figure 14:** Check for system issues. Here, user-initiated diagnostics reported that twoof the six enabled channels were detected to have no shaker connected (Cage 5 and 6).

# 4. File Information

*PiezoSleep* saves five piezo data files. The file with a base name (provided at the beginning of the data acquisition session) and the *bin* extension stores the raw data from the piezo sensors. The file with the ***Feat*** extension and ***_br*** padded to the base name stores the breath rates for all channels. The file with the *FeatVec* extension stores the feature vectors used to calculate the sleep statistics. The *FeatVec* file is used by the analysis program *SleepStats* developed by Signal Solutions LLC. These files are updated to the disk at least once every 2 seconds during the data acquisition session. Therefore, if the system crashes due to a power failure, data up to the point when the computer system failed is saved. Stimulation time and parameters (frequency, amplitude, duration) are stored in the file with the *stim* extension. Finally, the experimental configuration (DAQ, device, RatQwake configuration, etc) is stored in the ***_config.csv*** file. The program samples the piezoelectric signals at 120 Hz and 16 bits (2 bytes) per sample per channel. A 16-channel recording will write 332 MB of data per day. If disk storage is limited, it may be necessary to periodically stop data collection, remove existing data files, and restart the recording with a new file. This, however, complicates file and data management. It is best to have a computer with enough hard disk space and removable media to transport the large files to other systems for archiving and analysis. The size of the raw piezo data file can be estimated in terms of the number of days and active channels by the following formula:

$$B = \frac{(2*24*602*120)*d*c}{10^6} \approx 20.74 \text{ dC}$$

where *d* is the number of days, *C* is the number of active channels, and *B* is the file size in megabytes (MB). The ***Feat*** and ***FeatVec*** files are considerably smaller in size than the piezo *bin* file. The *Feat* file takes up about 0.34 MB/day, and the *FeatVec* file consumes roughly 1.4 MB/day for each channel, as compared to the 20.74 MB/day for the *bin* file. The size of the *stim* file will be much smaller, but the actual size will depend on stimulation settings and experimental conditions. The ***stim*** (*_stim.csv*) file contains information regarding stimulation applied to each cage. When any cage is stimulated, a row is added to the CSV, within which the

first column is the description of the event ('stim'), the second column is the timestamp of the event, and the subsequent columns are the stimulation parameters for the cages activated. If a cage is successfully activated, the corresponding column will contain the frequency, amplitude, and duration of the stimulation waveform, and will be formatted as 'freq/amp/dur' (e.g., '50/0.550/1' for 50 Hz, 550 mV, and 1 second). Columns corresponding to cages not activated will contain '-/-/-'. The stim file also contains an indication of stimulation faults, such as the 12Volt power supply not being turned ON. Also, when diagnostics is run by pressing the ***Run Diagnostics*** button, the status(0-5) of each stim device is written to the stim file. The legend is as follows: 0=Connected, 1=Disconnected, 2=ExternalShort, 3=InternalShort, 4=Cage Not Enabled, 5=12V Not Present. Finally, the ***config*** (*_config.csv*) file contains the settings used to configure the experiment. This file is automatically generated once the software startup sequence is completed and can be used as a template for future experiments where the same settings are desired (e.g., when restarting a recording after cage cleaning). The file can also be opened to determine what settings were used for a particular experiment (Note: If any parameters were changed mid-experiment, the content of the file will represent the last saved settings). When starting an experiment, if a *config* is loaded, the contents are displayed to the user before proceeding. Any changes to the settings will be stored in the new auto-generated *config* file (Fig 15).

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Data File: | C:\Users\SigSol\Documents\MyData.bin | | | | | | | |
| Number of Recording Channels: | 8 | | | | | | | |
| Device Information: | 32387056 | Dev20 | USB-6001 | | | | | |
| Light Dark Times: | 6 | 0 | 0 | 18 | 0 | 0 | | |
| Animal IDs: | Cage 1 | Cage 2 | Cage 3 | Cage 4 | Cage 5 | Cage 6 | Cage 7 | Cage 8 |
| Activation Mode: | 0 | 0 | 0.025 | 10 | 0 | | | |
| Randomization Range: | 20 | 127 | 0.05 | 3.65 | 0.5 | 2.01 | | |
| Activation Period: | 6 | 0 | 18 | 0 | | | | |
| Stim Cages: | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| Stimulation Parameters | | Frequency | Amplitude (V) | Duration (s) | | | | |
| | Cage 1 | 55 | 1 | Ext.Trig | | | | |
| | Cage 2 | 55 | 1 | Ext.Trig | | | | |
| | Cage 3 | 55 | 1 | Ext.Trig | | | | |
| | Cage 4 | 55 | 1 | 1 | | | | |
| | Cage 5 | 55 | 1 | 1 | | | | |
| | Cage 6 | 55 | 1 | 1 | | | | |
| | Cage 7 | - | - | - | | | | |
| | Cage 8 | - | - | - | | | | |

**Figure 15:** Example config file opened in spreadsheet editing software.

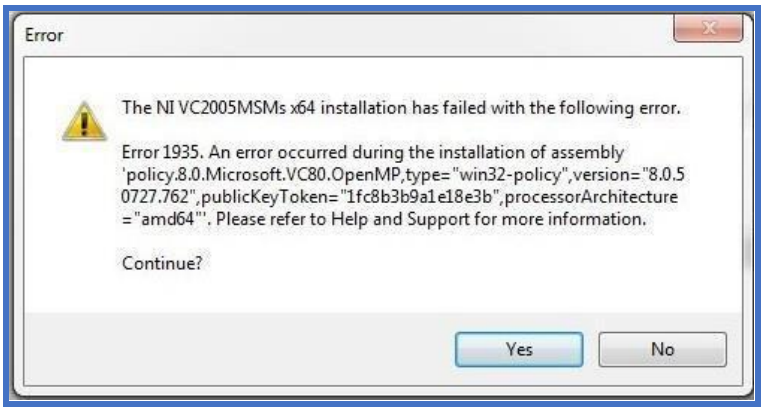
The contents of the config file are as follows:

| Row Number | Setting: | Description |
|---|---|---|
| 1 | Data File | Local path specifying data file location and name |
| 2 | No. of Recording Channels | Integer number of recording channels added |
| 3 | Device Information | ID No., Local Device ID, Model |
| 4 | Light Dark Times | Lights on Hour, Lights on Min, Lights on Sec, Lights off Hour, Lights off Min, Lights off Sec |
| 5 | Animal IDs | User Specified IDs for each cage (Default: Cage 1, Cage 2,… Cage N) |
| 6 | Activation Mode | Protocol (0 = Sleep Based, 1 = Activity Based, 2 = Manual, 3 = Open Loop),<br>Min Sleep Dur, Activity Threshold, Open-Loop Interval, Random Interval (0 = Specified Interval, 1 = Random Interval) |
| 7 | Randomization Range | Ranges used when randomizing Freq, Amp, or Duration. Freq (Min, Max), Amp(Min, Max), Dur(Min, Max) |
| 8 | Activation Period | Stimulation Start Hour, Stimulation Start Min, Stimulation End Hour, Stimulation End Min |
| 9 | Stim Cages | 0 = Stimulation Disabled. 1 = Stimulation Enabled |
| 10 | Stimulation Parameters | 9 x 4 matrix (including headers) containing stimulation parameters for each cage (8 cages will be shown even if < 8 cages were added to the recording. Any rows that correspond to cages > Number of Recording Channels will be filled with '-' |

# 5. Troubleshooting

**Troubleshooting errors during PiezoSleep installation or operation**


This error generally occurs when the software is being run from a user account different than the one it was installed using. Please switch to the user account used during installation of the 'PiezoSleep' software and try running the software again.




This error generally occurs due to the following two reasons:

1) Windows Update is running or has some updates pending.

2) The anti-virus software in your system is blocking the installation of “NI VC2005MSMs” because it sees it as a threat.

Please do the following:

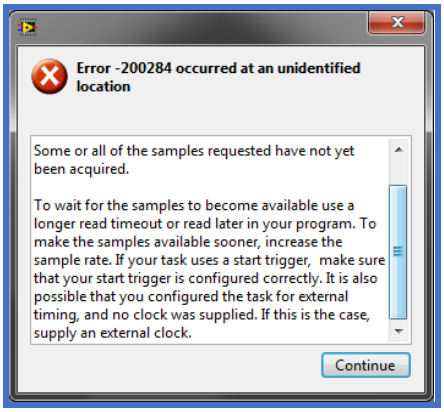
1) Turn off the anti-virus software and firewall temporarily.

2) Go to the system’s ‘Control Panel’ and check if there is any ‘Windows Update’ running or pending. If that’s the case, finish updating and then restart your system and try the installation again.

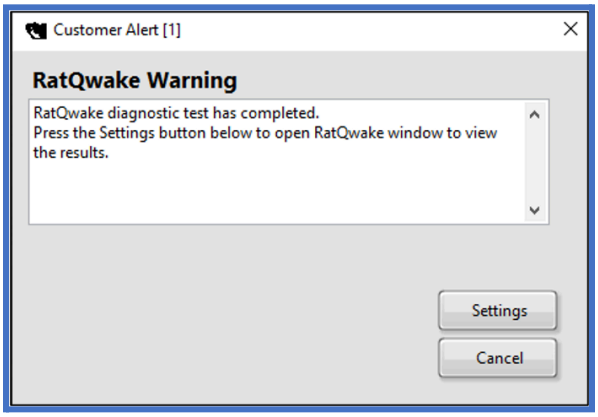
*Unable to locate the LabVIEW Run-Time Engine.*

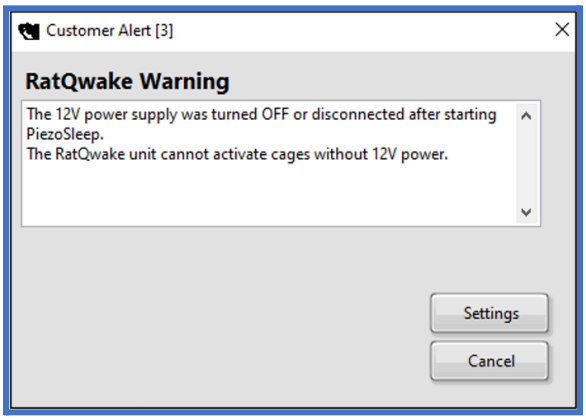
*PiezoSleep requires a version 2020 (or compatible) LabVIEW Run-Time Engine. To correct this problem, please contact the vendor of PiezoSleep or download the LabVIEW Run- Time Engine from the National Instruments website.*

This error generally occurs when there’s another version of LabVIEW installed on your machine, which hinders the installation of the LabVIEW Run-Time Engine 2020 (LV-RTE-2020). In this case, the LV-RTE-2020 will need to be manually installed before installing *PiezoSleep*. Please contact the technical team at Signal Solutions LLC for further help.


This error generally occurs when the USB port on your computer, which powers the DAQ, loses power (i.e., shuts down). Please refer to the section on Computer Settings of the manual and make the appropriate changes.




These are examples of warning messages that will be encountered when an issue is found from running a diagnostics check on cages with stimulation enabled. Pressing the Settings button in these windows will take you to the diagnostics screen. At the beginning of the experiment, a stimulation diagnostic check is automatically run on each of the enabled cages and will display a warning message if an issue is found. After the experiment has been started, most of the items tested by the diagnostics check must be initiated by the user clicking the ***Run Diagnostics*** button.