Webinar Q&A Report

Noninvasive, Automated Measurement of Sleep, Wake and Breathing in Rodents

Q: *How does the PiezoSleep system distinguish between inactivity (quiet wake) vs. sleep?*

B. O'Hara: The piezo sleep signal is characterized by a steady 2.5-3.5 Hz breathing feature, while quiet wake has additional small movements causing irregularities in the signal. These differences are included in the automated sleep/wake scoring algorithm. By visual observation, the PiezoSleep software routinely correctly identifies quiet wake as wake, such as when the mouse is sitting quietly, sniffing, grooming, etc. The automatic sleep/wake detection algorithm used in the SleepStats Data Explorer software has been validated with simultaneous EEG recording. Of 422,179 EEG epochs classified as wake by manual scoring, 92.4 % of piezo scored events agreed with EEG scored wake, and 7.6% were misclassified as sleep by the piezo signal. Similarly, of 373,642 epochs classified as sleep by human scored EEG, 91.4% were classified as sleep, and 8.6% were classified as wake by our algorithm. Precision and recall for sleep are 91.4%, precision for wake is 92.4% and recall is 92.3%. Our automated scoring is well within the range of inter-scorer agreement for manually scored EEG, reported between 91-95%, with substantial time and cost savings.

Q: *Can data be monitored remotely while an experiment is in progress (i.e. no need to physically be in the lab disturbing the animals)?*

B. O'Hara: Once mice are placed inside the cage with food, water and bedding, no monitoring is needed beyond the daily health checks. The PiezoSleep program can be monitored from any other computer using remote monitoring software such as TeamViewer, if there is internet access. Data can also be collected while an experiment is ongoing. PiezoSleep files can be remotely downloaded and analysed with SleepStats software with no interruption of the experiment. PiezoSleep will continue to collect data until the experiment is stopped either manually or remotely.

Q: *Is this system able to integrate with sleep deprivation chambers?*

B. O'Hara: We have integrated the PiezoSleep system with the Lafayette Sleep Fragmentation chamber to collect sleep information during a sleep deprivation experiment. The standard PiezoSleep monitoring system identifies episodes of sleep disruption, provides information on how quickly the mouse returns to sleep after the bar sweep, and collects all sleep parameters for the duration of the experiment from pre-sleep deprivation period through the recovery period. Post-sleep deprivation information such as percent sleep in the following hours, bout lengths, etc. provides information about the sleep-related effects of the sleep fragmentation. The PLUS systems (**Calamari Plus, Squid Plus**) provide the added capability of sleep-feedback control of the Lafayette system, such that the bar sweep is triggered only when sleep is detected (by a user-defined period of sleep). This additional feature would likely improve the experimental protocol by reducing stress to the mice, since they are not continuously forced to move while both awake or asleep. Currently, the system is compatible for sleep detection and/or sleep feedback control with singly housed mice.

Q: *Does the PiezoSleep system come in various sizes and dimensions to fit other sleep frag systems?*

B. O'Hara: Currently, a single sized piezo sensor is used.

Q: *How does your system differentiate between REM and NREM sleep? Is REM integrated in the quiet wake stage? Or is it integrated into the (NREM) sleep stage?*

B. O'Hara: Our current algorithm collectively calls REM and NREM sleep, as validated by EEG recording. The regular breathing associated with NREM becomes irregular in REM, but the body is relatively still and immobile compared to wake. The resulting quasirhythmic "piezo" signal differentiates sleep from quiet or active wakefulness with accuracy comparable to a human observer (Donohue et al. 2008).

Q: *Is external cage vibration an issue for this system? Do the cages need to be put on an "anti-vibration" table to avoid interferences?*

B. O'Hara: Normal static racks and cabinets are typically used to conduct sleep experiments, however for optimal results, the piezo system should be set up in a room that is as quiet as possible. In any new environment, a twenty-four-hour run with empty cages should be performed to determine whether any air movements or vibrations from vents, fans, machinery, other equipment, etc. are present that could compromise results. Any external signal could potentially mask or interfere with the biological signals from mouse activity. Facility HVAC systems often have blowers that come on at certain times of the day, and it is important to optimize rack and cage placement away from air vents to minimize air blowing into the cage and across the sensor. If strong air currents or vibrations are detected in an empty cage run, sensors can be shielded by re-positioning the rack, or physically blocking the air flow in the vicinity of the cages. In any experiment using sensitive equipment, the quieter the room, the better the results. Good practices for any sleep experiment include reducing disturbances from personnel doing routine checks, rescheduling other experiments in the same room to avoid waking mice during their normal sleep period (light period), making sure the timers for lights on/off are working properly, and other common sense precautions to minimize disruptions.

Q: *Is it possible to measure sleep, wake, breathing in group-housed rodents using the PiezoSleep system?*

B. O'Hara: Sleep/wake detection using the PiezoSleep system requires that mice be housed singly. Multiply housed mice will not all be awake or all asleep at the same time, and it is not possible to distinguish the activity of a particular mouse in group-housed mice.

Q: *Can this system distinguish between normal sleep versus cataplexy? Can it detect seizures?*

B. O'Hara: The piezo system has been used to detect seizures in a mouse pilocarpine epilepsy model. Seizures were detected with high sensitivity (around 90%) but lower specificity. The piezo system was used to identify spontaneous seizures after the latent period. Seizures are typically monitored by visual or video observation over a period of several weeks following pilocarpine administration, whose success rate for inducing epilepsy can be as low as 50%. The high sensitivity of the piezo detection may provide a more efficient screening method to identify epileptic mice prior to EEG implantation. The system has not been used to study cataplexy in narcolepsy mouse models in any detail, but as with seizures, there are likely to be characteristic signals that could be used as an initial screen for cataplexy. If you have a new application for the PiezoSleep system, please contact us for a discussion.

Q: *Is it possible to measure sleep pressure? As in sleep homeostasis, which increases after a loss of sleep. Can you measure sleep recovery?*

B. O'Hara: Increased sleep pressure is usually assessed by EEG delta power once the animal falls asleep. Our sensors do not detect this output. However, there is also usually increased sleep consolidation. Bout length measures, as one would expect, tend to increase with increasing sleep debt, but we have not fully quantified or validated this yet.

Q: *Could we apply the same technology for analysis of human sleep?*

B. O'Hara: Yes, Piezo sensors have been used to monitor breathing, heart rate and sleep in humans.

Q: *Do you have any example data of a mouse having sleep apnea?*

B. O'Hara: We have detected what look like apnea events in the breath signal output in mouse strains that have known apnea as part of the knock-out phenotype. Apnea-like events appear as brief pauses in the breathing signal during sleep, sometimes preceded by a sigh (a brief, high amplitude signal).

Q: *Can you provide information about where a system can be purchased and the cost?*

B. O'Hara: Please contact us for a quote on our website: http://www.sigsoln.com/contact/. Before contacting us, check out the sizes and features of our different PiezoSleep systems to estimate the best system for your needs. The number of mice to be monitored per experiment, as well as hardware requirements, determine the cost of a system. The smallest sleep monitoring system (the **Calamari**) can accommodate from 1-8 mice housed in our custom bottomless cages. Larger systems accommodate up to 80 mice (the **Giant Squid**). The standard hardware is for straight-forward data collection. The PLUS versions have capabilities to interface with other systems, for example, to provide sleep-feedback control of an input. If REM/NREM discrimination is an important aspect of your work, when the REM/NREM software becomes available, you will be able to add that capability to your existing system. If you have any questions, please ask on the contact form or give us a call. We love to hear what customers are working on, and would be happy to help with any experimental design or specific application questions.

Q: *Have you seen the recent paper of staging sleep just based on respiratory pattern from plethysmography (*[PMC5282481](PMC5282481)*)?*

B. O'Hara: Both the **PiezoSleep** system and plethysmography monitor sleep states using breath features that are specific to sleep and wake, and both are non-invasive compared to EEG recording. The pros and cons of each system should be weighed to decide which is more appropriate for a particular application. Plethysmography collects respiratory information over short periods of time, minutes to hours, whereas the study of sleep, by its very nature, benefits from longer periods of study. The main disadvantage of plethysmography is the inability to keep mice in the chamber long enough to collect adequate information about sleep parameters. Bodily functions such as urination compromise the output, so mice must be closely monitored for the duration of the experiment. There is no guarantee that the mouse will be asleep during the recording period, or, that the desired sleep related events will occur while the mouse happens to be asleep. The **PiezoSleep** system can and does detect many of the same respiratory outputs detected by plethysmography, including respiratory rate during sleep, sighs, and pauses in breathing such as occur in apnea. An advantage of plethysmography appears to be high sensitivity for REM sleep, although with manual scoring. The sleep/wake scoring in the **PiezoSleep** system is fully automated, but is currently limited to sleep and wake. For researchers where short periods of sleep are sufficient and who require REM/NREM discrimination, plethysmography may provide the answer. The **PiezoSleep** system provides high accuracy for sleep/wake discrimination over longer periods of time, at substantially lower cost.

Q: *Have you thought or tried to measure pressure changes in your cages to match with the piezo data - similar to the Buxco (now DSI) plethysmographs?*

B. O'Hara: We have tested the piezo sensor in a plethysmography chamber, and breath rates extracted from both signals correlate quite well while the mouse is asleep. Respiratory rate during quiet wake is also obtainable with the **PiezoSleep** system. When the mouse is active, other movements obscure the piezo breath signal. The piezo system was developed to provide a simple, inexpensive, high throughput method for sleep research, as well as a non-invasive, stress-free, more natural environment for the research animal. The procedure for using the **PiezoSleep** system is as simple as putting the mouse in the cage, adding nesting material for a good "night's sleep", and starting the software. Plethysmography is geared toward short term data collection and is more labor intensive and expensive but may provide additional respiratory information not detected by the piezo sensor.

If you have additional questions for Dr. Bruce O'Hara regarding content from his presentation or wish to receive additional information on measurement of sleep, wake and breathing in rodents please contact him by email:

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