Developing a Data Analysis Pipeline for Novel Bio-Logging Tools

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A Note on COVID-19

This thesis was completed during the 2020 COVID-19 pandemic which had a substantial impact on my experimental procedures and results. In forming my approach in early 2020 with my mentors, it was not yet clear that institutional shutdowns/slowdowns, mandatory distancing, travel restrictions for international students, and field research approvals would be impacted with such magnitude. Here, I include details with regard to our initial approach — including a major field component (Appendix) — in addition to the laboratory data we collected. With that, I hope the reader appreciates some of the rocks left unturned by the necessary pivot my work had to take in order to complete my project while meeting the high standards I asked of myself.

Acknowledgements

This thesis would not have been possible without the support of many people. I would like to thank my mentor, Dr. Matt Gaidica, and my PI, Dr. Ben Dantzer. This project, and the opportunity to even do an honors thesis, would not have been possible without both their help and guidance. Working on this project with them as part of Dantzer Lab has been an inspiring and rewarding experience. I would also like to thank Dr. Ada Eban-Rothschild for letting us record data from the mice in her lab, as well as Dr. Ines Sotelo for assisting with the procedure. Finally, I would also like to thank my family and friends for their constant support, encouragement, and (mostly virtual) company during long and often challenging writing and coding sessions, and most of all for believing in me as I ambitiously set out to complete this thesis in the chaos of a global pandemic.

Abstract

Bio-logging is a method of measuring and studying animal behavior. It involves attaching sensors directly to animals to record various measurements such as heart rate, acceleration, and electrophysiological data such as the electroencephalogram. This technology allows for the study of wild animal behavior in their natural environments, as studies have shown that their behavior in a controlled laboratory setting can differ greatly from their natural behavior. My research group has developed a novel bio-logging toolset that will aim to answer several important questions about behavior and physiology in wild animal models. My research here focuses on validating our bio-loggers and providing an analysis pipeline for future investigations using BORIS and MATLAB. The analysis pipeline I focus on developing in this project serves as a systematic method for handling data recorded from the bio-logger in the future. Specifically, I quantify behavioral observations from video in order to characterize how bio-logger data correlate with specific actions or motifs. These 'ground truth' data are foundational to our groups bio-logger development and quality assurance. Indeed, future research may have limited opportunities to directly observe animals, making the accuracy of the behavior-bio-logger relationships ever more important. My techniques also provide utility towards automated behavioral classification using supervised learning. I also highlight some challenges of developing such a data analysis pipeline. Finally, I outline a potential implementation of our bio-loggers in studying sleep behavior in wild North American red squirrels.

Keywords: bio-logging, electrophysiology, behavior, BORIS, EEG, delta power, sleep

Developing a Data Analysis Pipeline for Novel bio-logging Tools

"It clearly emerged that electrographic recordings without simultaneous behavioral observations may be of limited value in the examination of sleep duration. However, since the criteria for defining behavioral sleep are often inconsistently applied, a purely behavioral characterization of sleep in the absence of electrographic correlates may also lead to unreliable or misleading information." ~Claudio Stampi (1992), Why we nap

Bio-logging is a method of studying animal behavior by collecting data directly from the animal through the use of attached sensors that record parameters such as heart rate, electrophysiological data such as the electroencephalogram (EEG), and multi-axis body acceleration. Bio-loggers allow for remote observation of wild animals where direct observation may be impossible, such as an animal living in an inaccessible habitat (Rutz & Hays, 2009). They also make it possible to study elusive animals, where previous attempts may have been inhibited by a lack of encounters (Wilmers et al., 2015).

Although studies can be done in laboratory settings, such data may not reflect the animal's natural behavior. Being housed in the laboratory eliminates many natural behaviors, such as foraging for food or fighting for territory. For example, EEG data revealed that the brown-throated three-toed sloth (*Bradypus variegatus*) slept for a significantly shorter duration in the wild than when in captivity (Rattenborg et al., 2008). The laboratory is often designed to be as controlled as possible, which is a stark contrast from the unpredictability synonymous with nature (Calisi & Bentley, 2009). Taken together, behavioral data collected in a laboratory is simplified and lacks the complexities of what we would observe in a natural environment. Getting an accurate view of an animal's natural behavior could drastically improve our understanding of their natural biological and physiological responses.

Another advantage of using bio-loggers is the ability to observe animal movement patterns without the physical strain of following the animal around. In fact, bio-loggers can record data at high frequencies, capturing minute details that may be invisible to the naked eye (Whitford & Klimley, 2019). Animals may also change their behavior in the presence of a perceived predator or even a human observer, resulting in bias (Nickel et al., 2021). Using an accelerometer overcomes this challenge by providing a constant record of where the animal goes, with minimal impact on its behavior (Patterson et al., 2019). This is especially useful in recording nocturnal animals, where visibility and time constraints may hinder physical tracking. For example, a novel combination of tri-axis accelerometers and acoustic recorders enabled the successful study of free-ranging snowshoe hares (*Lepus americanus*), a nocturnal and cryptic species (Studd et al., 2019a).

Bio-logging has been revolutionary in the field of ethology, but it comes with its own set of challenges. The data it records is useful only if we know what the numbers mean. Direct observation is still key to relating our recorded data to real-world behaviors (Altmann, 1974), and we can draw that link only if we know what the animal was doing. Behavioral observations also help to validate data, and may even be more accurate in classification than bio-loggers in some cases. Studd and colleagues, 2019b found that the classification accuracy of their decision tree for the full observation period was as low as 26.8% for complex behaviors such as running, with an overall classification accuracy for all behaviors of 70.6%. When using a separate data set of 5 minute observation periods, they managed to obtain 100% classification accuracy for travelling, and 96.4% accuracy overall for all behaviors. The large difference in accuracy highlights the importance of taking behavioral observations into consideration, and not relying purely on bio-logger data alone.

Additionally, with the sheer number of options for sensors available, it may be difficult to decide which combination of tools would be optimal for the purposes of the study (Williams et al., 2020)**.** Having the wrong understanding of a sensor's purpose or using one without fully understanding how it works could lead to imprecise results, especially if the data is not reviewed and verified (Whitford & Klimley, 2019). There are also limitations to the detection abilities of a sensor. While they can detect subtle electrophysiological differences, they may not be able to detect subtle behaviors. In Studd and colleagues (2019a), the accelerometers could not detect chewing in the snowshoe hares as the acceleration generated by the behavior was not high enough for it to be measured. Taking all this into account, behavioral observations are important for calibrating bio-loggers and verifying their accuracy (Studd et al., 2019b)**.**

The present study focuses on validating the novel bio-logging toolset developed by my research group, as well as providing an analysis pipeline for handling future data. This pipeline would serve as a protocol to ensure consistency in the way data is analyzed. We obtained recordings from laboratory mice to test the device. I logged behavioral observations from video recordings using Behavioral Observation Research Interactive Software (BORIS), an open-source behavioral analysis software (Friard & Gamba, 2016). To understand the electrophysiological manifestations of the observed behavior, we used custom MATLAB scripts to plot data recorded from the bio-loggers. To check for the consistency of bio-logger readings, we tested the specific null hypothesis that readings for the same behavior between both days would not have any significant differences. To validate that our device was recording EEG signals accurately, we tested the specific hypothesis that delta oscillations (0.5-4 Hz) – typical of NREM sleep (Panagiotou et al., 2017) – would be significantly higher during observed periods of sleep than in any other behavioral state.

Materials & Methods

Recording Electrophysiology in Laboratory Mice

Subjects

We used female C57BL/6J wild-type laboratory mice (n = 2, ~12 months old) for our experiment provided by the Eban-Rothschild Lab. These mice were previously used for recording electroencephalography (EEG) and were thus outfitted with skull head caps (i.e. implants) that exposed connections to the prefrontal and parietal cortex and one bipolar connection for electromyography (EMG) from the neck muscle. The implants were performed by a skilled technician and secured to the skull using stainless steel screws and dental cement. Though we did not take part in these procedures, they were approved by the University of Michigan IACUC.

Bio-loggers

We recorded EEG biopotentials using a custom bio-logger device that was fabricated in-house. The bio-logger recorded EEG and EMG at 250 Hz and 3-axis accelerometry at 25 Hz to onboard memory, after which the data downloaded and archived on a computer.

Procedure

A GoPro HERO5 camera was mounted on the cage and angled to record the full view of the cage. The camera was set to record at 30 FPS. It began recording and was placed to capture the moment the bio-logger was initialized, such that the two datasets could be synced post hoc. The bio-logger was attached to the mouse head cap by a skilled technician and placed into a cage that was mostly empty with the exception of bedding material and nesting material to promote sleep. The cage used for recordings was a familiar environment as it replicated their home cage.

During the recording, the lights remained on since mice are nocturnal sleepers. Mice were left to freely behave for one hour.

Analysis

Behavioral Observations

A total of two sets of data were collected from separate recording sessions. The first was collected on January 8, 2021 (Day 1) and the second on March 11, 2021 (Day 2). 88 minutes of video were recorded on Day 1 and 32 minutes were recorded on Day 2. The videos were imported to BORIS to assist with demarcating behavioral events. Briefly, we uploaded our behavioral videos to the software, created an ethogram by mapping computer keys to behaviors, and then coded our observations as the videos played. Behavioral observations coded included Wake-Still, Walking, Head Movement, Sleep, and Twitch. The twitching behavioral state was to account for any sudden movements the mouse made when it was in any state of presumed behavioral quiescence (ie. Sleep and Wake-Still)..

The behavioral time points were then exported from BORIS into a CSV-file, which was then imported into MATLAB and used to extract relevant epochs of bio-logger data. Using a custom script (https://github.com/debho/mouse-ephys), we also generated a plot from this data (Figure 2), which was a visual representation of what behaviors took place at each time point (1-second resolution). Accurate and synced time points allowed us to correlate behavioral states with electrophysiological data.

Binning Behavioral Observations. We divided our data into 5-second bins to facilitate our statistical analysis using a custom MATLAB function. This created ‘independent observations’ of equal size that we could use for comparing means between days and between

behaviors. Figure 3A shows the overall plot of binned behaviors from Day 1. Figure 3B shows a zoomed-in view, where each dot and line pair represents a 5-second bin of data.

EEG Data

The electrophysiological data was analyzed using custom MATLAB scripts. Data was adjusted to remove outliers for more accurate calculation of mean powers. This involved using the *rmoutliers()* function to identify the indices of outliers (defined as a value more than three scaled median absolute deviations), and then using the *nearest()* function to replace those values with the nearest integer that would fit the dataset.

Raw EEG data from bio-loggers was used to generate power spectra with the *pspectrum()* function using a Fast Fourier Transform (FFT) of each of the 5-second bins. The power spectra showed power against frequency. Power represents the magnitude of neural activity occurring at a particular frequency.

The *pspectrum()* function with the *'spectrogram'* option was used to generate spectrograms from data. The spectrograms showed the power of each frequency at each time point.

Statistics

Recordings of the same behavior between different days were compared to check for consistency in the bio-logger recordings. To test the null hypothesis that there were no differences in recordings between days, we ran two-sample t-tests using the *ttest2()* function with Bonferroni-Holm corrections comparing the same behavior between the two days.

We also did comparisons using two-sample t-tests between behaviors for each day. The behaviors we chose to compare are Sleep and Wake-Still, as these behaviors are most pertinent to our future research interests that are centered around the neuroelectrophysiology of sleep.

Through this, we determined if behaviors were different from each other and where the significant differences were, if any.

Results

Spectrograms

By superimposing the behavioral observation plot onto the spectrogram, we were able to see the power at various frequencies against time, as well as what behavioral state the mouse was in at the time (Figure 3). Based on our hypothesis that delta power is associated with NREM sleep, we expected to see higher power in the delta band during sleep.

Day 1

The spectrogram showed patterns that were congruent with the patterns of our behavioral observations (Figure 4, top). Similar power-frequency patterns appear consistently with coded behaviors, which lets us draw conclusions on which signals each behavior produces. We also observed the presence of delta-alpha oscillations during Walking. This is something that we did not expect as both delta and alpha (8-13Hz) (Kamal et al., 2017) are not oscillations usually associated with locomotion (Buzsáki et al., 2003).

Day 2

Similar to Day 1, our comparison of the behavioral observations with the spectrogram showed that the behavioral data is mostly aligned with the electrophysiological data (Figure 4, bottom). However, we did observe the presence of delta-theta waves in some regions of Wake-Still, which is surprising, as signals at those frequencies are conventionally associated with sleep.

Statistical Analysis

Between Days

We found that there were significant differences in the low frequencies (Figure 5B and D) typically involved in NREM sleep.

Between Behaviors

Since we found that there were significant differences in recordings at low frequencies between both days, we did not combine the data for between-behaviors analysis comparing Sleep and Wake-Still data. Based on our hypothesis, we expected delta power to be significantly higher during Sleep than during Wake-Still.

There were significant differences between Sleep and Wake-Still for Day 1. Surprisingly, there did not seem to be much difference in delta power, and the parts that were significantly different seemed to be mostly at higher frequencies. For Day 2, Sleep and Wake-Still were significantly different, especially at lower frequencies (Figure 6). These results support our hypothesis that delta power is significantly higher during Sleep than during Wake-Still, as this was the case for most frequencies. This is despite the fact that some periods of Sleep were mistakenly coded as Wake-Still, which could have resulted in the analysis between the two states showing them to be less different than they actually are.

The difference in results between both days could be due to the differences in recordings between both days that we observed earlier in Figure 5.

Discussion

In this study, we aimed to validate the accuracy of the bio-logging toolset developed by my research group, as well as provide a future analysis pipeline for collected data. Using BORIS and custom MATLAB scripts, we ran analyses on the electrophysiological data and correlated it

with the behavioral observations. We tested the reliability and consistency of the bio-logger's readings by comparing electrophysiological recordings of the same behavior between data from two separate days. We also hypothesized that delta power correlated with observed sleep behavior, and we tested this by comparing different behaviors within the same day.

Although all EEGs were sampled at the same frequency (250 Hz), the number of data points collected by each of the bio-logger contacts differed. While this issue was resolved by using a custom MATLAB function to equalize the sizes of the vectors, it is still something that resulted in complications during data analysis. In addition, in our analysis of Day 2's data, we found that the actual sampling rate deviated from 250Hz and it was closer to 243Hz. While we were able to adjust for it manually in our code, this further highlighted the importance of running such verification tests on bio-logger devices, as well as implementing a rigorous syncing methodology in order to ensure precision in future data collection.

Interestingly, Sleep and Wake-Still seemed to have more points of significant difference in the data from Day 2 as compared to Day 1. There are a number of possibilities for this difference. For one, we used different subjects between both days, and individual differences between the mice and the recording conditions could have contributed to the observed differences. The recordings for both days were taken at different times of the day. As such, the mice could have varied in levels of sleepiness, which has shown to alter levels of NREM sleep, which would manifest as altered levels of delta wave production (Kim et al., 2020). The mice may have been at different phases of their estrous cycle at the time of recording, which affects their levels of stress and anxiety – mice exhibit lower levels of anxiety during the high-estrogen phase than in the low-estrogen phase (Jaric et al., 2019). This would have resulted in them experiencing different levels of stress at the time of recording, and stress has been shown to

result in altered sleep physiology in mice (Olini et al., 2017). We could have been comparing measurements from slightly different brain regions, or electrode quality could also have differed between subjects. It is also of note that the electrode implant was accidentally detached from the mouse's head during the removal of the bio-logger after the recording on Day 2, suggesting that the implant could have been loose. Loose implants affect signal readings (Mishra et al., 2018), which could account for some of the variability. Finally, the differences in theta power (6-8Hz) in Wake-Still between both days could have been caused by the mouse in Day 1 scratching its head more than the mouse in Day 2 – it has been observed that laboratory rodents tend to scratch in a way that produces signals in this particular frequency band (Kadam et al., 2017). With this in mind, we could account for this in future studies by adding Scratching behavior into our ethogram for behavioral observations. Furthermore, there were also updates and refinements made to the bio-logger software between the first and second recording session, and this could also account for some of the differences we observed. Nevertheless, this highlights the importance of testing and verifying such devices and performing data analyses as a part of an agile development pipeline.

We also observed the presence of delta-alpha oscillations during Walking (Figure 4, top). This is an unexpected observation, as we would expect to see theta oscillations during locomotion (Buzsáki et al., 2003) instead of delta or alpha. As mentioned earlier, delta oscillations are characteristic of NREM sleep. However, we also noted that the period of Walking happened right before sleep, and alpha activity is present in waking behavior prior to the onset of sleep (Achermann, 2009). This could potentially explain why we saw such a pattern. While we are unsure of the exact reasons behind this observation, this highlights the importance of validating electrophysiological data with behavioral observations. If we had based our analyses

solely on bio-logger data and not correlated it with behavioral data, we may have assumed that the mouse was in NREM sleep during that period purely due to the presence of delta waves. The fact that our data analysis pipeline enabled us to identify this shows that it has tremendous potential in guiding and directing our interpretation of our data and not falling prey to relying solely on "common knowledge" about data patterns.

Limitations

Behavior logging has inherent limitations. There is the possibility of error due to human reaction time. With a sampling rate as high as 250 Hz, even the slightest time offset can result in misalignments between the behavioral observations and the electrophysiological data. This would preferentially affect brief, ballistic movements and subsequent peri-event analyses.

Additionally, it has been observed that sleep-wake behavior animals in captivity may not always reflect their actual brain states (Rattenborg et al., 2017). For example, in Day 2's spectrogram (Figure 4, bottom), the presence of delta-theta waves at the regions of Wake-Still directly before and after the block of Sleep revealed that the mouse was likely asleep during those time points, and not awake like it was observed and coded. While we could improve our ethogram to better define behavioral states, such as defining sleep as a continuous inactivity that lasts for 40 seconds or more, (Pack et al., 2007), it is still challenging to be completely accurate in coding behavioral observations.

These limitations highlight the importance of having effective testing and validation protocols in place for the development of such devices. Through working on these limitations, we also found ways to further improve and optimize the bio-loggers and our handling of the data.

Future Work

The development of a data analysis pipeline and the identification of ways to further refine our data collection and handling are just the first steps in furthering the development of my research group's novel bio-logging toolset.

Bio-logging can be further enhanced with the use of supervised learning to train machine learning models to extract behaviors from raw data. There are some existing tools that have been developed for this purpose. For one, the AcceleRater is a web application designed to train machine learning models for supervised learning of various behavior modes using accelerometer data collected from bio-loggers. The models trained by the AcceleRater performed well, with the mean accuracy among all the models being decently high at 81.51% (Resheff et al., 2014). Such results are promising for supervised learning-trained models in future developments in the field of bio-logging. With future improvements, it would be possible to train a machine learning model with supervised learning to generate an ethogram from the bio-logger data.

We would also like to revisit the possibility of recording animal behavior in the wild. Our original project set out to record wild North American red squirrel (*Tamiasciurus hudsonicus*) behavior by installing nest cameras into their dreys to capture footage of their in-nest behavior, so that we could study their sleep-wake behaviors and look for any variability in their sleep patterns (Appendix). This project was hindered due to unforeseen circumstances brought about by the COVID-19 pandemic, such as a delayed start on data collection due to the increased time taken for processing our facilities use request. This left us with insufficient time and opportunities to collect any substantial data before in-person activities were suspended for the remainder of the Fall 2020 semester after the Thanksgiving break. The inclement weather conditions past that time period would also have been less than ideal for field work that involved

climbing trees and spending prolonged periods of time outdoors. We would like to return to recording animal behavior in a natural environment, this time by attaching the bio-loggers onto wild, free-ranging red squirrels.

Conclusion

In this study, we aimed to verify the bio-loggers as part of the development process, and at the same time, develop a data analysis pipeline for data recording in the future. Through the present study, we highlighted areas of improvement for the further refining of the bio-logger software. We successfully developed a data analysis pipeline that proved to be very helpful in guiding our interpretation of data by revealing interesting patterns that we may have missed otherwise, as well as identified ways we could further tweak our analysis, such as refining the ethogram to record behaviors that could potentially account for unusual readings. The results of the present study show that there is great potential for the future development of these devices. By continuously working on fine-tuning both the bio-logger software and our analysis methods, this novel toolset would really assist my research group in their quest to answer various questions about wild animal behavior and physiology.

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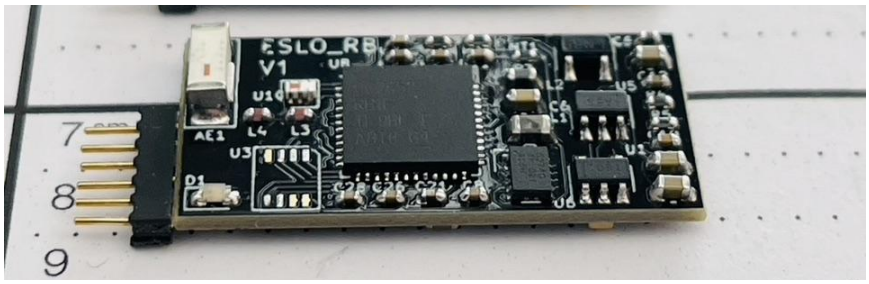
Figure 1

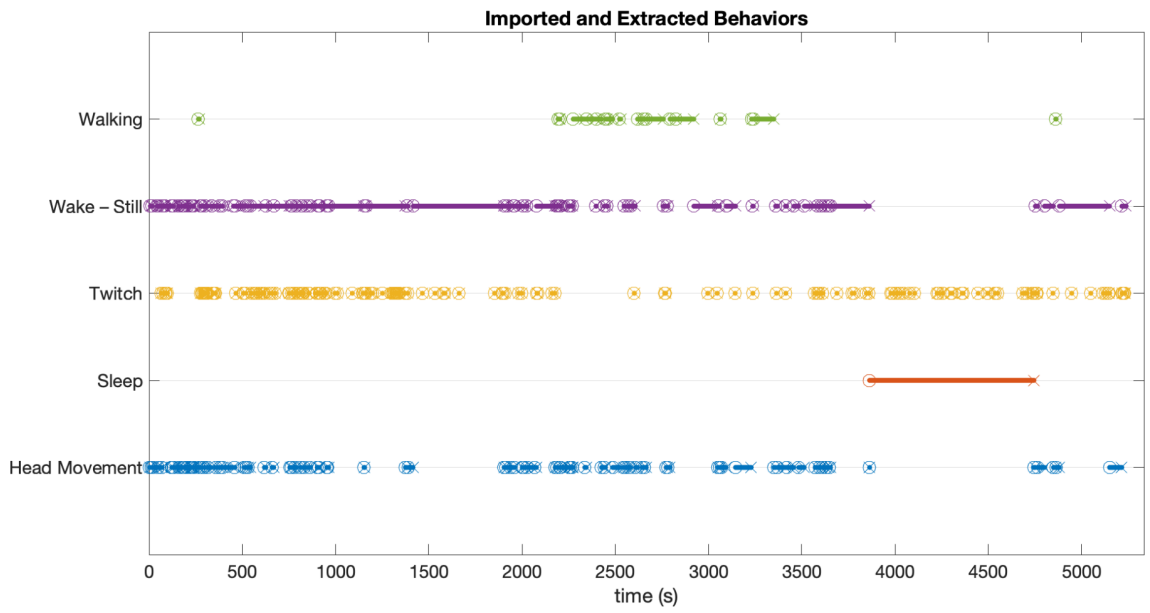
Bio-logger Device



Novel bio-logger devices developed by my research group that were tested in this study. Devices are capable of recording 4 channels of bipolar biopotentials, accelerometry, and transmit data over Bluetooth Low Energy.

Figure 2

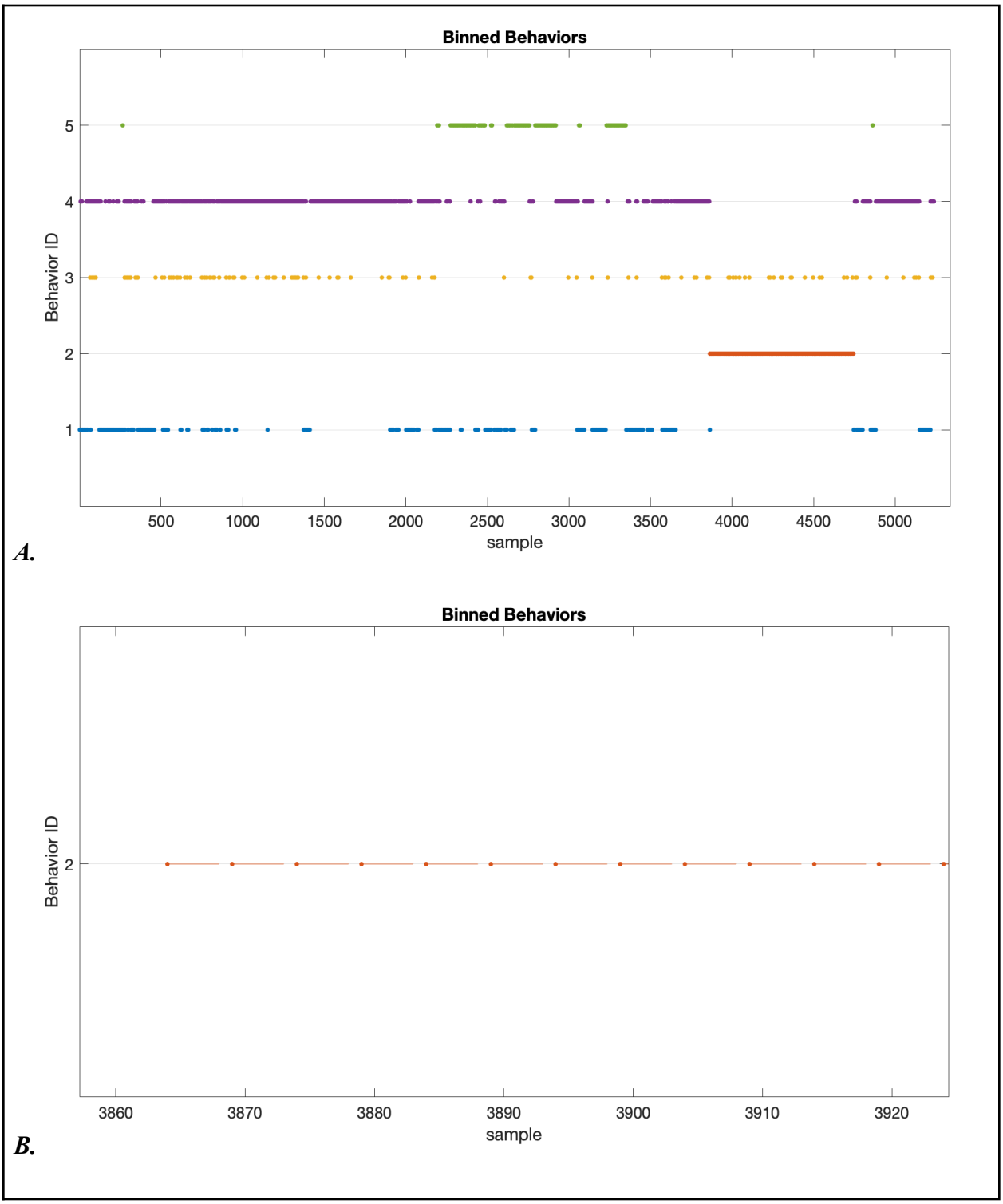
Binary Plot of Behavioral Observations



Behavioral observations against Time from footage recorded on Day 1, generated by a custom MATLAB script from observations manually logged using BORIS. The beginning of each behavioral event (except for Twitch) is denoted by 'O' markers while the end is denoted by 'X' markers. Instead of spanning a duration, Twitching occurred discretely.

Figure 3

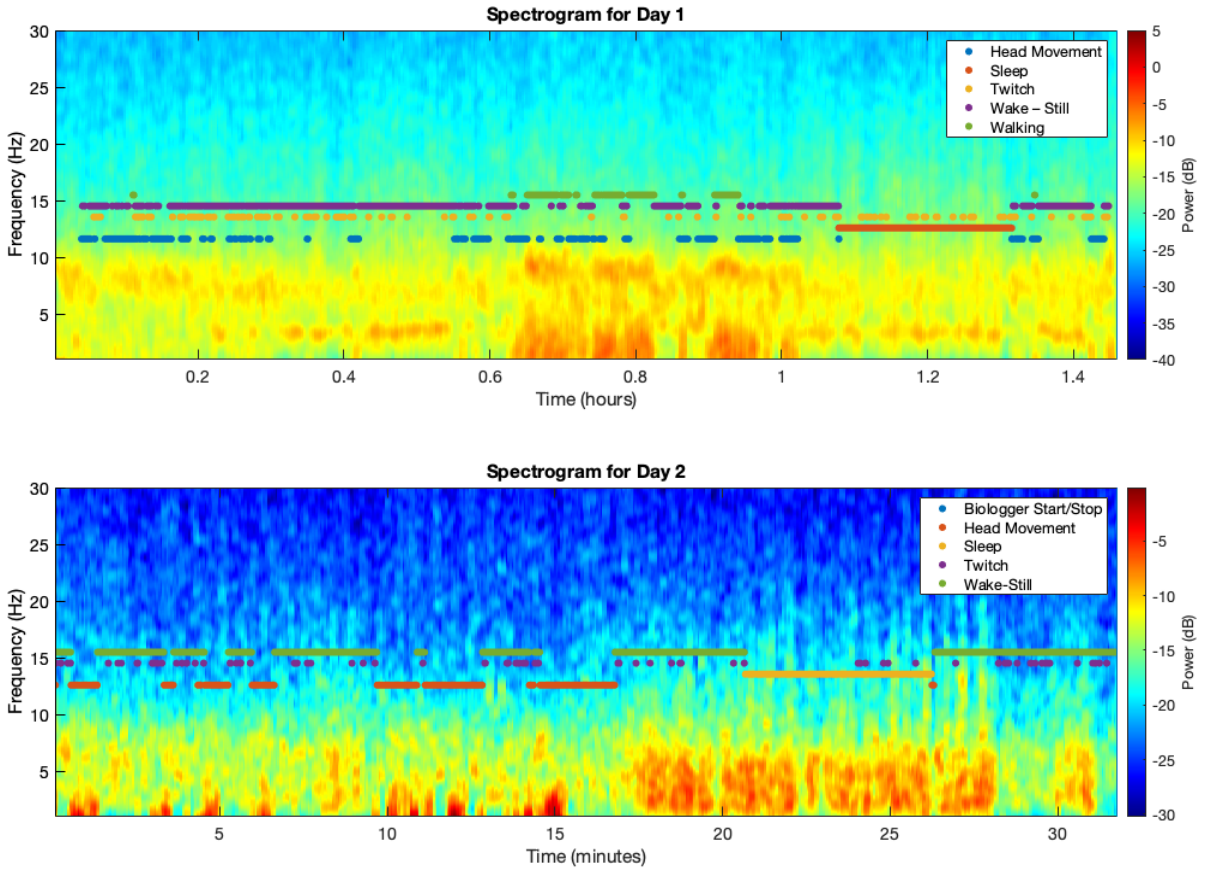
Example of Binning Procedure



A. The dots represent the start of the 5-second bins corresponding to ongoing EEG. *B.* A zoomed-in view of the plot. Each dot and line pair represents one 5-second bin.

Figure 4

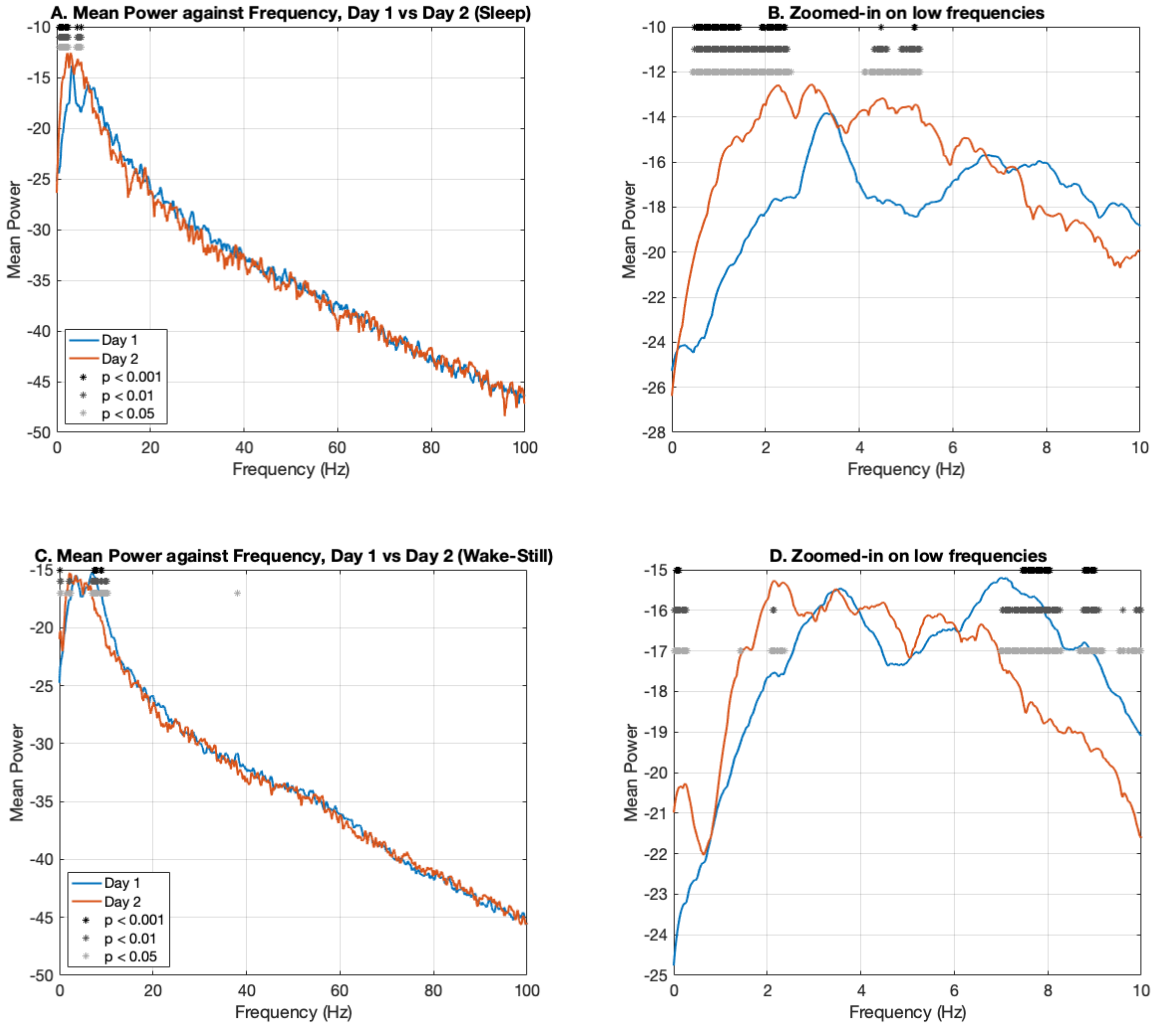
Spectrograms



Superimposing behavioral plots on spectrograms makes it possible to look at what signals each behavioral state produces and vice versa. The colorbar scale (*caxis*) was adjusted empirically.

Figure 5

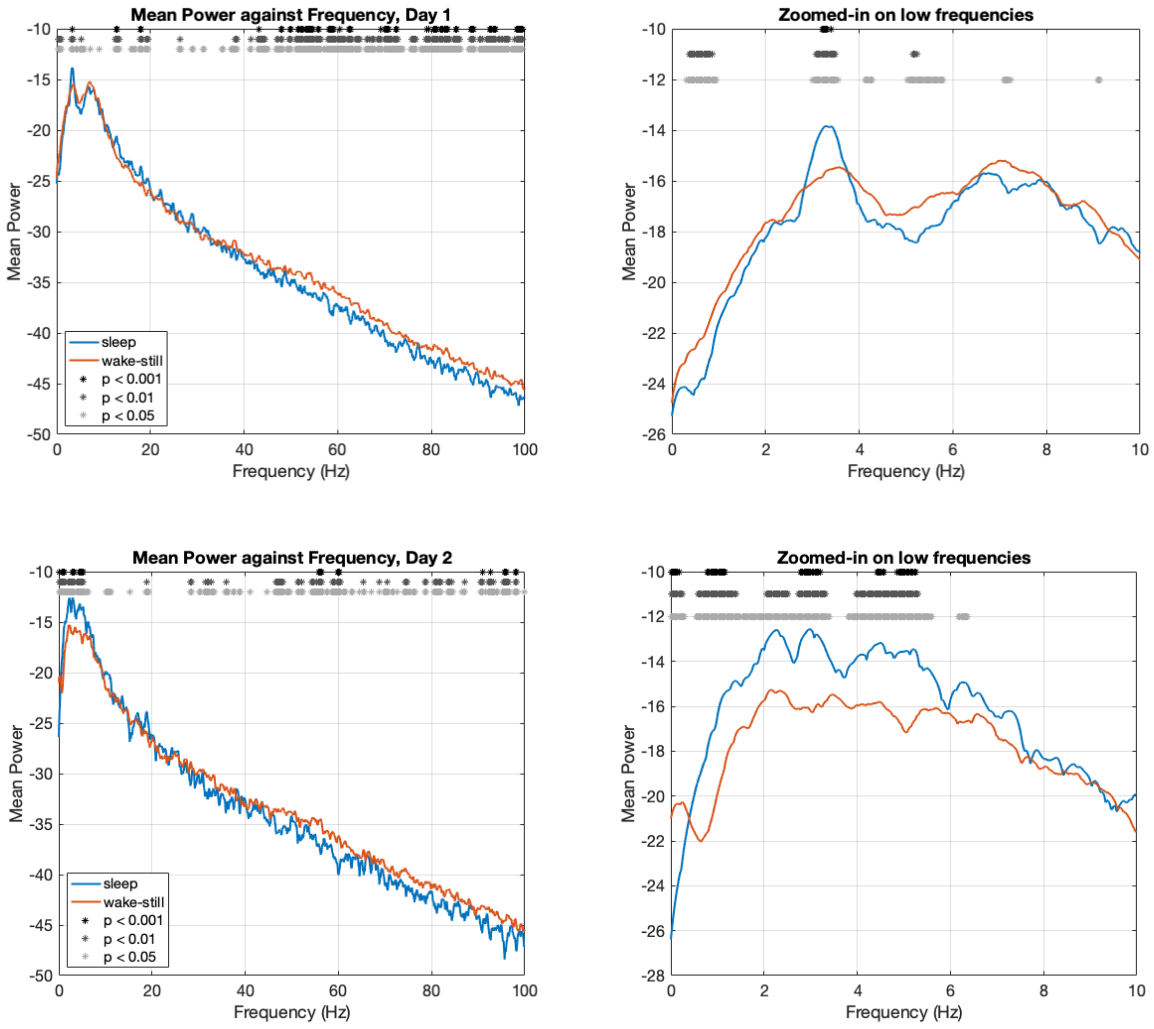
Power Spectra Testing Bio-logger Consistency



The mean power against frequency for bio-logger recordings from Day 1 (blue line) were compared with recordings from Day 2 (orange line). Sleep (A and B) and Wake-Still (C and D) were compared. The asterisks on the top of the figures indicate whether the recordings were statistically significant from each other at different significance levels: 0.05, 0.01, and 0.001, represented by different shades of gray from darkest to lightest, respectively.

Figure 6

Power Spectra Comparing Behaviors Within the Same Day



Mean power was plotted against frequency for Sleep (blue line) and Wake-Still (orange line) for both Day 1 (Top) and Day 2 (Bottom). The asterisks on the top of the figures indicate whether the behaviors were statistically significant from each other at different significance levels: 0.05, 0.01, and 0.001, represented by different shades of gray, from darkest to lightest,

respectively.

Appendix

Capturing Video of Wild Red Squirrel Nests

Location and Subjects

We conducted our research at Saginaw Forest, a University of Michigan (U-M) field site about 4.4 miles west of campus. Field research and travel were approved by LS&A. We were approved to experiment with animals through a Michigan DNR Scientific Collectors permit and all matters relating to animals were approved by U-M IACUC through protocol PRO00009223.

Climbing and Field Safety

Prior to carrying out any fieldwork, we attended a one-day climbing clinic at Peabody Ice Climbing to learn basic climbing safety and skills. These skills included belaying, tying basic knots, and climbing with and without a partner. We developed a climbing protocol to ensure consistency and safety in our climbs. We established a field safety protocol that covered all other potential issues including infectious zoonotic disease risk, injury reporting, and handling other environmental hazards such as inclement weather. At least one member was trained on First Aid and CPR.

Apparatus

For video capture, we used a snake-style, digital camera with infrared lights inserted into the drey. We waterproofed the nest camera by inserting it into an empty syringe and filling any gaps with fast-drying silicone. We used a digital video recorder with a 512GB SD Card, powered by a 38,400 mAh battery pack. To protect the rest of the apparatus from environmental conditions, we put the digital video recorder and battery pack into a 10L dry bag (Figure A1).

A. 

B. 

C. 

Figure A1. Nest camera set-up for recording in-nest video footage of red squirrels A. PVC connector inserted into an opening in the red squirrel drey. B. The waterproofed nest camera inserted into the drey. C. The set-up used to record video. The screen showed images from the nest camera as guidance for insertion into the drey and the rest of the electronics were kept in

the dry bag for weather protection. This set-up was left tied to the tree for the duration of recording the video footage.