Webinar Q&A Report:
Harnessing EEG Methods to Improve Basic and Translational Research for CNS Disorders

1. **In your experience, what are the biggest challenges for measuring or analyzing EEG?**

   Dr. Robert Gould: As I eluded to in my presentation, choosing the proper sampling time (both duration of sampling and time of day to sample) is one hurdle. Data analysis is another large initial challenge, in part because of the large amount of data collected.

   Quantitative EEG analysis really needs to be completed in a state-dependent manner (pulling qEEG out during epochs of just WAKE, NREM or REM stages) and then binning them. Finding an efficient and accurate way to do this can be overcome with custom coding or free MATLAB software, but can be a challenge if you aren't fluent with MATLAB or other coding programs.

   Sleep analysis is also time consuming if done manually. This can be a bottleneck in the data analysis pipeline (e.g., you can't pull out state-dependent qEEG until after sleep staging is complete). Again, it depends on the research question. For example, shorter sampling intervals or keeping animals awake via various repeated stimulations can reduce the need for sleep staging.

   For someone without surgical experience, the implantation may seem like a large challenge, but can easily be overcome. DSI offers a 1-2 day surgical tutorial if needed. Sleep staging can be time consuming if done manually. NeuroScore software does offer automated sleep scoring capabilities as well as consultation to aid and develop data extraction/analysis.

   Michael Girand: It might go without saying, but it is important to stress that the quality of data is dependent on the surgical approach and the devices being used… Good data in, good data out. However, once beyond that, perhaps the biggest challenge is data management. When recording large amounts of data, it is important to utilize the tools and resources available to you. Researchers should take advantage of both DSI's Technical Support and Data Services teams. Both groups have a tremendous amount of experience with large datasets, unique study design, and analysis.

2. **Do you have any tips or considerations for research that requires the measurement of multiple biopotential signals simultaneously, for example measuring respiration along with EEG?**

   Dr. Robert Gould: I don't have direct experience with multiple measurements, so my advice is contact DSI. Equally important, find another researcher conducting similar multiple measurements and see if they are willing to share their advice. I have been eager to share my experiences and advice because it took me a while to learn it, and because many other researchers were eager to share their knowledge with me. Lastly, DSI has a large database of users. They can (and often do) reach out to other users and see if they are willing to be contacted for advice (like myself).

   Michael Girand: Combining applications is being requested more often. There are a few options. The Ponemah software platform can synchronize wireless signals from telemetry with what we call hardwired approaches. So, you can combine telemetry with whole body plethysmography, for example.

   If many biopotential signals are requested, we can turn to a tethered EEG approach whereby we work with Plastics One to incorporate electrical commutators and tethers, allowing us to obtain several biopotential signals. This tethered approach can also be used in DSI's whole body plethysmography chambers.

3. **What other physiological endpoints can be monitored using DSI technology and synchronized with EEG data?**

   Michael Girand: DSI's Ponemah software platform is flexible enough to accommodate several signal types and is not limited to telemetry implants. More popular signals include both cardiovascular and respiratory endpoints, including left ventricular pressure, blood flow, respiratory tidal volume and rate. In addition, relevant information from 3rd party equipment can also be collected. Examples include activity wheels, treadmills, optogenetic pulses, gas analyzers, and behavioral inputs. These are just a few examples.

   See the following link for additional information:
   https://www.datasci.com/products/signal-conditioners-and-amplifiers

4. **What are some advantages and disadvantages of wired vs. wireless EEG?**

   Dr. Robert Gould: Wireless EEG provides an opportunity to record from a freely moving, uninhibited animal in multiple settings (homecage; operant chamber, novel environment, etc.). Wireless recordings may provide an opportunity for longer durations of recordings (e.g. 24hr or longer) that may be limited by tethered designs. One possible disadvantage of wireless recording is ensuring that the signal is not disrupted by other electrical sources.

The disadvantage of a wired EEG is that sleep may not be as "normal" because of the pressure on the skull possibly minimizing the normal posture of the rat; wired recordings may produce more artifact associated with jostling of the tether. Using a wired set-up eliminates the need for a battery (and may also thus be more cost effective, long-term) and may be paired with other neuroscience techniques (optogenetics, site-specific recordings). Wired set-ups may reduce time and invasiveness of surgery.

Michael Girand:

Wireless:

- Fully implantable, reducing animal staff and enabling more natural behavior
- Reduced stress approach
- Small to large animal
- Chronic data collection
- 1-4 electrode pairs
- Bandwidth: 0-5 -200 Hz
- Sampling rate: up to 1000 Hz
- Ponemah is capable of both wireless and wired EEG collection approaches

Wired (tethered):

- Small to large animal
- Acute data collection
- 1-12 electrode pairs
- Bandwidth: 0.05 – 1 kHz
- Sampling rate: 20 kHz/channel
- Ponemah is capable of both wireless and wired EEC collection approaches

5. Can you provide an overview of sleep analysis capabilities within NeuroScore?

Dr. Robert Gould: NeuroScore is a flexible software program to allow basic visualization as well as basic data extraction capabilities. NeuroScore does have an automated sleep scoring algorithm – I like to have my eyes directly on the data so I have not used this, though.

I will briefly describe some of the functions I use NeuroScore for, but I recommend contacting DSI for a program brochure. NeuroScore can be used to visualize all channels recorded from DSI transmitters (EEG, EMG, temperature, activity, battery life, signal strength, activity measures; likely pressure sensor data as well [no experience to confirm]). While I have not personally done this, it can import other signals, to sync with these signals.

With regard to sleep analysis, a user can designate an epoch length (5 or 10 sec or at user discretion) and then toggle through each epoch and assign a pre-defined stage (e.g.

Wake, REM, NREM, artifact). There is also an option to define one's own states; Shortcut keys can be assigned each stage.

Regarding EEG signal, NeuroScore has the capabilities to derive/transform signals from the raw EEG (signal filtering, thresholding, Fast Fourier Transformations, Root Mean Square, ratios of one frequency band to another, any many more) as well as basic analysis (max/min/median/ave for any signal). Neuroscore can allow visualization of the Spectral Periodogram as well. All these aspects can help identify sleep stages.

NeuroScore has template data reports as well as the capability for user-defined reports to be designed and saved for future use. Template reports can generate the duration of time within a given recording period (or subset of that period) in each sleep stage, number and average bout lengths, etc. Additional tables can be made and exported for future analysis/compilation for any signal at any designated epoch duration (e.g. delta power across 5 sec, or 1 hour averages).

So, in short, many very useful, powerful tools are available to aid data extraction and management. Of note, several analyses are not currently included: 1) the ability to only pull EEG data from within a designated sleep stage (e.g. delta power ONLY from NREM sleep stages); this has to be done from another data platform (excel, MATLAB, Python, etc.); 2) no ability to average data from multiple different recordings or animals. However, I have found this to be a very powerful software platform for EEG analysis. NeuroScore is also capable of importing multiple data file types from other sources.

Michael Girand: Automated rodent sleep scoring is based on the frequency content of the EEG and presence of EMG activity and movement. Stages include. Paradoxical Sleep, Slow Wave Sleep (SWS-1, and SWS-2), Wake, and Active Wake. Automated large animal sleep scoring is based on the American Academy of Sleep Medicine standards for human sleep scoring. The algorithm uses EEG, EMG, EOG, and activity data. Stages include REM, Non-REM (N1, N2, N3), Wake and Active Wake. Automated rodent sleep scoring is also available. We advise overreading the data manually in both large and small animal applications to verify the algorithm is scoring data to your standards and specific criteria.

6. **Is NeuroScore an alternative to other data analysis tools such as MATLAB? Can you provide a brief description about the different capabilities between the two.**

Dr. Robert Gould: See description above regarding NeuroScore capabilities. Many people use MATLAB and free or modified EEG analysis modules. I have not used MATLAB so I can't speak directly to its function. For strictly qEEG analysis, MATLAB is highly capable. I would imagine sleep scoring could also be done with MATLAB. I am beginning to learn Python coding to assimilate group qEEG or sleep data AFTER I've completed sleep scoring and extracted individual data from NeuroScore.

In its basic form, sleep stages are determined based on relative prevalence/distribution

of power in different frequency bands. It can almost be broken down into an if:then hierarchy. [If presence of activity=active wake; if no activity = not active wake, then look at EMG. If EMG amplitude is high = quiet wake, if no, not quiet wake, then look at EEG for sleep…etc]. I'm sure this is the premise of an EEG toolbox in MATLAB and designated specific ratios of high: low power and delta: theta power. But NeuroScore is easy for a non-coder to use (like me).

Michael Girand: - Yes, NeuroScore is a software platform offered by DSI to assist with analyzing neuroscience telemetry data and it also assists visualization of EEG and EMG data along side other parameters such as cardiovascular if desired. Ponemah software can export to both NeuroScore and MATLAB. The intuitive interface within NeuroScore allows for easy analysis of the frequency content, sleep scoring, and spike detection (for seizure applications). The NeuroScore program has been relied upon from researchers within the neuroscience community for years. DSI cannot speak to EEG analysis capabilities of MATLAB. Our data services team does not rely on it for EEG interrogation for our customers.

Contact DSI to learn more about NeuroScore!

7. **Is there a difference in using wires in contact with the dura versus screws directly suspended over the brain?**

Dr. Robert Gould: I don't have enough experience to comment or distinguish one way or the other based on electrical conductance [or other]. What I can say is that when I was initially validating EEG methods, we tried both wires and screw methods in mice. Qualitatively, we did not see a difference. Based on my understanding, screws should also be in contact with the dura. So, when posed with the question "wire around screw and screw touching the dura, or wire directly touching dura?" I made the decision that the screw was simply one more step in the surgical process and removed it. Perhaps a screw would increase surface contact and generate a larger signal for a longer period of time? However, I've been able to record for over a year in the same rats and ~6 months in mice using wires only.

Michael Girand: There are many considerations when selecting the right implant and method to implant leads for your EEG study. Please visit DSI's Knowledge Base for more information: https://support.datasci.com/hc/en-us/articles/360026487593-Guidelines-for-the-use-of-EEG-Screws-with-Telemetry

8. **What are the prospects of sleep linking to pain or pain-related disorders?**

Dr. Robert Gould: As I'm not an expert in pain or pain-related disorders, I would defer to a literature search. My hunch is that acute and chronic pain may be associated with different types of sleep disruptions. There is definitely a clinical literature (less so in

animal studies) describing analgesics on sleep. I would image there would be a lot of opportunities to pair current pain research with EEG to look at both sleep impairments, but also qEEG as perhaps an indirect measure for pain or alleviation of pain.

9. Is it possible to analyze rodent behavior based on EEG without using video recording?

Dr. Robert Gould: I think the answer to this question depends on what "behavior" is to be measured. Seizure detection can be done without video, although some researchers really prefer to be able to visualize these full seizure activity as well; EEG can detect some seizure activity that is not picked up by video.

Sleep staging can be determined without video. However, using EEG only as a surrogate measure for another behavior (cognitive, aversive, stress, etc) would be difficult to know exactly what is occurring with ongoing behavior, in my opinion. I could imagine characterizing a behavior with video and EEG and then confirming from EEG alone that a similar predictive EEG signature correlated with a behavior. Thus, EEG could then be used as a surrogate measure of a behavioral state (e.g. stress response).

Michael Girand: In my opinion, improving confidence in results by synchronizing video data to physiologic signals should be considered when building a robust study design. Video-EEG is particularly helpful when confirming seizure-associated behaviors, and confirming whether the subject is in a paradoxical or active wake state.

10. How do you identify different stages of sleep from EEG data?

Dr. Robert Gould: In short, different sleep stages are characterized by different ratios of oscillatory activity that can be readily identified by visual inspection. Different patterns of oscillatory activity are a result of different firing patterns of neuronal populations that underlie the transition and maintenance of different sleep states (see Brown et al cited below).

For example, Slow Wave Sleep is associated with a higher prevalence of delta power and lesser ratio in higher frequency power. Rapid Eye Movement sleep is associated with lesser percentage of delta power and a higher relative percentage of theta power and higher power (e.g. gamma). A number of automated algorithms (one in NeuroScore) do this based on rations of delta/theta in addition to activity and EMG activity. A few citations that describe this in detail are below:
Brown et al., (2012) Control of sleep and wakefulness. *Physiological Reviews* 92:1087-1187. doi: 10.1152/physrev.00032.2011

Representative traces are also included:
Ivarsson et al., (2005). Antidepressants and REM sleep in Wistar-Kyoto and Sprague-Dawley rats. *Eur J Pharmacol* 522:63-71. doi: 10.1016/j.ejphar.2005.08.050

Representative traces are also included in the Supplemental Material of:
Gould et al., (2016). State-dependent alterations in sleep/wake architecture elicited by the M4 PAM VU0467154 – Relation to antipsychotic-like drug effects. *Neuropharmacology* 102:244-253. doi: 10.1016/j.neuropharm.2015.11.016

Michael Girand: Supporting material can be found here
https://www.datasci.com/docs/default-source/default-document-library/sleep-stages-classification.pdf?sfvrsn=bb91e265_0

https://www.datasci.com/resources/blogs/publication-reviews/2019/03/04/understanding-the-mechanisms-behind-sleep-dysfunction-critical-to-overall-health

11. Can you briefly describe the surgical process for telemetry implantation?

Dr. Robert Gould: I can refer to several published descriptions of the surgery including the one below:

Gould et al., (2016). State-dependent alterations in sleep/wake architecture elicited by the M4 PAM VU0467154 – Relation to antipsychotic-like drug effects. *Neuropharmacology* 102:244-253. doi: 10.1016/j.neuropharm.2015.11.016

In brief, under anesthesia, animals must be prepared for aseptic surgery (shaving the implant side and skull; cleaning with approved anti-septic scrub [betadine/EtOH or chlorhexidine solution]). Ideally, animals are placed in a stereotactic frame for securing the head in place and identifying electrode placements. After the animal is prepared for surgery, an approximately 1-inch incision is made dorsomedially along the thoracolumbar region (for subcutaneous implant) or along the ventral midline (for intraperitoneal placement). The connective tissue that attaches the skin and muscle is blunt dissected apart on one side to allow for the sterile transmitter to be inserted. The leads will then be passed underneath the skin toward the neck for EMG and EEG lead attachment. A 1-in mid-sagittal skin incision will be made to expose the skull and dorsal neck muscles. ~ 1 cm piece plastic sheath is removed from the EMG leads. These exposed wire leads are then inserted through the neck muscle, perpendicular to the muscle fibers. ~ 5mm of this plastic sheath will be placed over the distal end of these EMG leads such that no wire is exposed to the skin, and will be secured in place using silk thread. Holes are then drilled in the skull, without puncturing the dura. EEG leads are placed in contact with the dura

and the leads will be permanently affixed to the skull by quick drying dental acrylic. The skin is then sutured with 3.0 vicryl sutures ensuring to leave enough slack in the lead lines for the animal to move/sleep comfortably. Pre-, peri, and postoperative analgesia should be administered andor antibiotic regimens should be considered. Given longitudinal nature of these studies, all precautions to maintain health and well being should be taken.

Michael Girand: please contact DSI at support@datasci.com

12. How long can you use telemetry implants in a single animal?

Dr. Robert Gould: I can't say definitively. I can say I've kept the same transmitters in rats for over a year, and in mice ~ 6 months. Depending on outcome measures, sleep patterns and duration do change with aging, so take this into consideration.

Michael Girand: The use of telemetry implants and the length in which they can be used in a single animal depends on a few variables. Outside proper animal health and well-being, transmitter life is dependent on battery life. Researchers can turn off devices when not in use, saving battery life. When it is time to collect data once again, the transmitters can be turned back on.

The warranted 'on-time' battery life for DSI transmitters ranges from 1 month to 4 months. However, based on study design and transmitter usage, transmitters are often in animals ranging from months to years. Specifications for small animal transmitters can be found at https://www.datasci.com/products/implantable-telemetry/small-animal-telemetry

Contact Information

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