Webinar Q&A Report:

Advancements in Wireless Technology for Single Unit Electrophysiology Recording

1. **Can the Cube2 be adapted for mice?**

   No, Cube2 cannot be adapted for use in mice. However, we have launched an independent solution for small animal telemetry applications, based on the same technology and architecture as Cube2, called "SAT2" (Small Animal Telemetry 2). Currently, the SAT2 implant weighs ~5.2 grams, with half of that weight being allocated to a battery which supports 1.5 hours of recording (16 channels @ 20 Khz). As of January 2018, we are completing final testing on this platform. For those interested in learning more, please contact us at info@neuralynx.com

2. **What is the failure rate for a single unit recording (single neuron, not ECoG or field recording) in deep nuclei, such as substantial nigra or hypothalamus? How long will a standard recording last in a freely moving condition?**

   The use of the Telemetry system has very little impact on how long you can "hold" a single neuron. The recording quality of deep structure neurons is more dependent on the electrode and implant type. Therefore, we do not see any particular reason, or reasons, why a single neuron could not be "held" for long duration.

3. **In your experience, do animals acclimate better to the wireless systems compared to the tethered systems?**

   The consensus seems to be that rodents do show more natural behavior with the Cube2 system as opposed to tethered recording setups (during acclimation and experimentation?). We attribute this to the total weight of the neural recording system and associated strain that can occur from tethered solutions, including the fact that the tether pull force is not consistent but is based on the animal's location from the center of the maze. For instance, the total weight of a microdrive plus the Cube2, capable of recording up to 64 channels for 35 minutes simultaneously, is ~29 grams. In comparison, the Hyperdrive and HS-54, used for the NASA/NIH Space Shuttle NeuroLab project, weighs ~36 grams.

4. **You mentioned that you record for 6 hours. Do you save continuous data, spikes, and/or spike waveforms?**

   K. Hoffman: The Cube, like the Digital Lynx SX, sends only raw unfiltered, continuously sampled data to the Cheetah Software. The Cube2 does not perform spike detection, that is done in Cheetah. When recording to the internal SD card, Cube2 raw data files are created. Once recording is complete, the files are offloaded to a PC where they are converted to CSC data. This CSC data can then be used to extract spikes. Therefore, the user can record continuous data for 6 hours (or more depending on batteries and uSD memory card size) and access all spike waveform data offline. NOTE: Neuralynx is currently working on a conversion utility that will transfer .crd file information directly to Matlab.

5. **Can you shut down specific (unproductive) channels to save data on the SD card?**

   Yes. We have a "Channel Masking" function that disables channels from being transmitted and written to the SD Memory card. This feature helps extend the recording capability per experiment and ensure data management is most efficient for the end-user.

6. **Can your dump the SD using WiFi?**

   It is theoretically possible, but it requires too much time (longer than the original recording time). The SD Memory Card is usually changed when the batteries need to be changed, therefore, changing the SD card manually at this time is the logical and most efficient process.

7. **Is Cube2 reusable? Is it easy to attach and remove?**

   Yes, the Cube2 and its accessories (battery, AFE, etc.), are reusable. The base configuration of the Cube2, which includes 64 channels, utilizes our patented QuickClip connection. This allows for easy connects and disconnects as well as providing long term connector durability. It is removed from the subject when recording is completed.

8. **Are there any other equipment needs for the data recording for Cube2? for example, the data receiver, A/D.**

   The physiological signals are digitized at the headstage – on the subject in the Cube2. These signals are either transmitted in real-time to our Cheetah software, or written to a local microSD card. The Hardware required to get started includes the Cube2, Access Point (WI-FI Router), Cube2 Starter kit, batteries, battery charger, and computer. We can also connect the access point into our Digital Lynx SX/SX-M Data Acquisition System. The Cube2's connector will connect directly to a Neuralynx EIB for tetrode recordings or we can adapt to a various number of connectors to record from a desired implant.

9. **For wireless data transmission, what is the distance limitation?**

   The Cube2's transmission range is up to 10 meters, depending on environment. The data from the headstage is a reliable transmission to the system's access point. This data is viewable in real-time and recorded with our Neuralynx Cheetah software.

10. **When you were doing the recording, was there a reference so you can compare the signal integrity with wireless data transmission? (data reference wired vs wireless)**

    K. Hoffman: There are two ways of interpreting the term "reference" from this question, so multiple answers are given. If referring to the electrical reference necessary for recording any biopotentials: selection of the reference is very important when using the Cube2, as with any recording system, since it is a digital headstage, and the desired reference signal needs to be hard-wired into your electrode input board and ultimately, into the Cube2. For example, if you wish to use an animal ground as reference, this would need connected/wired into the designated reference input for the Cube2 headstage. So, it requires a little advanced planning. But like with any biopotential recording, assuming a good reference signal is present, the wireless recording quality is identical to wired. If "reference" signifies how the wireless data quality compares to wired, there is no difference in quality, assuming good electrode connections. One of the advantages of Neuralynx's setup is that it's possible to record from both analog, wired headstages and the Cube2 simultaneously, in case you wish to view an overt, side-by-side comparison. In fact, this was done when the Cube2 was initially tested in the lab, and there was no qualitative difference between the wireless and wired.

11. **Does the wireless module support fully duplex data transmission (given that you will use stimulation + recording scenario)?**

    Yes, the Cube2 communications is fully bidirectional through the use of WiFi. We currently allow commands to be sent to the Cube2 for configuration commands and to set Digital I/O bits. We will soon be able to drive our new Fiber Mounted LEDs (FLEDs) with a new tiny module to drive the FLEDs. These commands are available over NetCom also for closed loop experiments.

    Please contact sales and support to discuss your experimental needs. Full complex stimulation will be available in future Cube product releases.

12. **What is the total bandwidth of the wireless system and what is the additional power consumption of the wireless system? Also, what is the range?**

    The Cube2 is a wireless headstage that records from up to 256 individual electrodes, - ±5mV Input Range, 80dB Common Mode Rejection Ratio (CMRR) at 60Hz, 2.5µVRMS Noise (0.1Hz to 9kHz), and 30KHz Sampling Rate.

    Neuralynx offers three standard battery options for the Cube2 with custom options available:
    - Battery Small (Type C)(~23 min Tx, 30 min microSD): 6.9 grams
    - Battery Med (Type A)(~1 hr Tx, 1hr20min microSD): 15 grams
    - Battery Large (Type B)(~2.5 hr Tx, 3hr15min microSD): 30 grams
    - Battery custom 18650 (~5hr15min Tx, 7 hr microSD): 45 grams

    Cube2 also has the option to write data to an on-board microSD card. This feature will increase your battery runtime up to 30% if you choose not to transmit data to your computer in real-time or limit the transmit time. The Cube2's transmission range is up to 10 meters, depending on environment.

13. **Are there any plans for bidirectional wireless communication (for closed-loop experiments)?**

    Because we use WiFi the data link is bidirectional. This allow commands to be sent to the Cube2 for configuration and to set Digital I/O bits. These commands are available over NetCom for closed loop experiments such as Optogenetics LED control with our new Fiber Mounted LEDs (FLEDs), current stimulation or other control such as drug delivery, audio or visual stimulation. Please contact our sales and support staff should you be interested to discuss this application further: info@neuralynx.com

14. **What wireless ranges are available?**

    The Cube2 range is dependent on several environment factors such as metallic structures and other WiFi channel activity. On our website, you can see the "Cube2 on a Spear" video which has the Cube2 moving very fast on the tip of a KungFu Spear over a range of about 10 meters. This is an open space with very little other WiFi data activity. Minimum range is specified at 4 meters, which allows for an 8-meter diameter maze. Open or unused WiFi channels can usually be found with a simple cell phone "WiFi channel analyzer" App which allows the best range and data rate performance.

15. **How many animals can be recorded from simultaneously? In what instances does the data drop out/lose signal?**

    Currently up to two Cube2s can be recorded from simultaneously when using our Digital Lynx SX to a single host PC, or when recording directly to the Cube2's internal microSD card. The WiFi transmission with the radios we use is very robust, allowing up to a 10-meter distance between the Cube2 and wireless Access Point, thus signal loss is usually not an issue. The only instance where dropout can be an issue is if recording in an environment where a large number of competing wireless networks are present.

16. **What is the error rate of wireless transmission?**

    As long as a valid connection between the Cube and the wireless AP is in place, the error rate of transmission should be close to 0. To minimize error rate, use a wireless channel that does not have competing WiFi networks. We've viewed recording sessions of several hours where no loss of connection, or "dropouts," occurred. In optimal conditions the dropouts should be very close to 0%.

17. **Can wireless technology better record cell activity during epileptic seizures?**

    J.A.Wolf: Generally speaking, it's difficult to record unit activity during seizures to begin with. To this date (Dec 12, 2017), epileptic activity has yet to be recorded from the pigs. Hypothetically, isolating unit activity in a wireless recording system should be enabled because there are no tether artifacts that may be present in a wired system. The one caveat is that if possible, you should have a priori knowledge of the rail-to-rail voltage range measured from the epileptic activity, to ensure that it won't be larger than the voltage range permitted by The Cube2 headstage (+/-5mv).

18. How do you attach the Cube2 on a daily basis? How "off the shelf" is the large animal solution?

K. Hoffman: The QuickClip connection used on the "off the shelf" Cube2 allows for it to be connected and disconnected very easily, not only on/off the same animal, but allows the Cube2 to be used across multiple animals at any one time. The Cube2 itself is usable right away. However, there are two aspects that require advanced planning and design before implementing the Cube2:

- The implant itself. The standard design of the Cube2 is the Neuralynx Quickclip, thus there might need to be a customized adapter that allows interface with the implant. The Cube2 headstage can be customized to utilize Omnetics connectors, and a design is being worked on that will allow different headstage connector types to be interchangeable.

- With large animals, there must be consideration for protecting the Cube2 itself. Neuralynx consulted with John and ultimately built the enclosure system he currently uses for recordings.

If you have additional questions for Neuralynx regarding content from their webinar or wish to receive additional information about their products and services, please contact them by phone or email:

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