"Functional Microdissection of a Hippocampal Circuit with High-Density Optrodes" – Webinar Q&A

1. **How many times do you need to clean the probe in the acute preparation?**
    a. You can clean the probes every day after the experiment or on different days, it doesn't matter. I recommend cleaning them frequently by submerging them in the enzymatic solution because that will prolong the life of the probe.

2. **How do you know the precise location of the probe in the brain?**
    a. If you are working in the hippocampus this is more or less direct because we learn to infer the position from the local field potentials and the information we get from the recording. You can use florescent dye on the probe so that they leave a track, but you will never be able to see exactly where the tip of the probe was. You will see where the channel was going through, following the track, but not so precisely. I recommend learning to rely on different signals to try and localize yourself.

3. **Are sites and LEDs functional all the time or do they tend to fail overuse?**
    a. That may happen. These probes, the substrate is silicone and therefore they are fragile, you may break a shank. For instance some of our probes only have three shanks but you can still use it if one shank is gone. If you aren't careful and apply a large current you can get in trouble with the LED. We haven't seen any electrode or LED go away except in one weird case. They usually behave properly. Basically when you buy the probe they are tested for quality.

4. **What is the maximum ripple frequency that is considered normal during a sharp wave?**
    a. Ripple frequency is between 100 or 200 Hz, but the more we play with the ripples we see that there is not a particular frequency there is a range. Depending on how you identify ripples. They are typically detected by thresholding. What you typically do is estimate the amplitude in 100 to 200hz and then you look for the peaks, and that limits the type of events you are recording. A normal ripple is between 90hz to 200hz, but you may have some slower or faster. And then pathological ripples tend to be faster but not all the time, you may also have low frequency ripples, you need to learn from a combination of signal features whether it's normal or not.

5. **How likely are you going to disrupt (damage the surrounding) cells when you dip the probes in the brain tissue?**
    a. If you penetrate slowly you don't damage that much. Typically you penetrate slowly and allow tissue to stabilize. Never start recording immediately after penetration, allow some minutes, typically 15-20 minutes, depending on how long you can wait in your preparation to stabilize. If you penetrate several times along the same place then you damage. You always leave a mark, even if you are skilled, you somehow still leave a mark, but the cells are still healthy.

6. **When recording with the microLED probes, did you do any channel-specific referencing?**
    a. No, we record against the ground, so we have the reference and the ground as the same. Signals in the hippocampus are typically recorded against an occipital screw implanted in the animal neck, over the skull.

7. **Does the plating procedure on the probe affecting in any way the LEDs?**
    a. We use the probe as we receive the probe from production. We don't plate the electrodes after, just use them as they are. You do not really need to gold plate them.

8. **Do you know how the impedances change after repeated acute use?**
    a. Impedance changes if you have deposition of whatever biological material, or whatever thing stuck in there, but as I said, if you clean the probe back to normal you see them working properly. If you take care of them properly, they work well.

9. **Say, when you are recording neurons in CA1 region. Given that neurons are tightly packed (with minimal empty space between two neurons), do these shanks rupture/penetrate any neurons?**
    a. You go blindly, you penetrate the region, you see them firing when you reach the stratum pyramidale then they calm down and you collect the sharp wave and then you know where you are. During the acute recording you don't see any particular change, there is some drift if the preparation is not stable enough, mechanically stable. This may happen some times in the headfixed, you fix the skull but the brain moves within the skull. If you are careful with the dura and with the fixation system, you don't have much drift. Within an hour that's okay, over an hour you may have some drift starting but Kilosort 2 can correct for that.
    Note added over transcription: We have never seen intracellular signatures with the µLED probe indicating penetration of the neuron. Note the probe dimension is much larger.

10. **I have not seen such strong cross shank inhibition with a camkii promoter. how much of the inhibition do you think is due to direct stimulation of interneurons with your different opsin promoters?**
    a. I think this is why we are still troubleshooting the differences between these different mouse lines. Within that specific opsin there is some crosstalk so we are losing specificity, also we think that somehow there is disinhibition because the microLED, even if we go with a very low amplitude current we see huge responses. This is why we prefer to go with the calbindin to be sparser in terms of expression. You can also control expression by diluting the titer of the titer of virus.

11. **How big was your craniotomy and for how long was your recording stable?**
    a. That really depends on the experiment. You can be very very tiny and open a small craniotomy, which will be very good to facilitate recording stability, etc. But as you see the distance between the shanks is 250 microns, that means that you need a craniotomy that accommodates the four shanks. If you lose a shank, we can do a smaller craniotomy, you can adapt. This is why the head prep is good. You can also open several craniotomies, so that you can move around.

12. **Which features do you use to differentiate physiological from pathological ripples if they are in the same frequency range?**
    a. Whether the animal is epileptic or not, but this is naive answer. If you are blind to the animal there are other features that allow you to know, for instance in the epileptic animal most of the sharp waves are very high amplitude, you have much more synchronization of signals firing and less specificity for firing during the ripples. If they are strongly pathological you cannot sort properly, this is a major problem because you have the spike collisions and the ripples are mini populations of spikes and therefore you stop seeing the cell. Many people get confused and think that during these pathological events neurons are silent, but what is actually happening is that you cannot sort. So there are many features of the event that you can use, the amplitude of the sharp wave, we like to couple the recordings with linear probe recordings, to know also about the sharp wave and the stratus radiatum, because these probes are only targeting the stratus pyramidale. A combination of features, amplitudes, shapes, slopes, synchronization, etc.

13. **Can you distinguish calbindin positive pyramidal cell responses from calbindin positive interneuron responses during optostimulation?**
    a. Yes and No. You can an adenovirus for the cre sequence with the CamKii, but the problem in our hands is the expression is going to CA2. We are estimating how many calbindin positive interneurons we have along the shank. They are not quite as populated by pyramidal cells. So we try to establish clear association between interneurons in the neighborhood of the penetration to estimate whether we are targeting them or not.

14. **Is there a need to plate the electrodes during different recordings?**
    a. No, we don't have the need to plate it. If you take care of the probe they are working as perfectly as the first day. The only reason we would change the probe is if they break.