

## **“Microcircuit Interrogation with Neuron-scale Optogenetics” – Webinar Q&A**

- 1. Have you noticed a significant effect caused by the latency of the microLED ramp time?**
  - a. From the time your signal generator outputs the voltage how long does it take the microLEDs to light? That is essentially so short you can dismiss that delay, the LEDs light in a time scale much faster than a physiological time scale. I haven't been able to measure it but it's in the hundreds of microseconds at most, it doesn't come into play in terms of how fast you can stimulate.
  
- 2. The amount of power measured from the microLEDs is small compared to optic fibers (e.g. uW from the LED compared to mW from the fiber). Does this affect the behavioral response?**
  - a. So optogenetics is used for many things at very different scales, if you're looking to control the behavior of an animal, the early class experiments stimulated dopamine neurons with optogenetics and showed that you can get place preference and stuff like that. That's not what the microLEDs are designed for and not their forte. Obviously if you want to turn on all dopamine neurons in the brain or light up the whole hippocampus and turn the whole thing on or off you would need an optic fiber to do that. In reality, if you have a light source like the microLED close to a neuron, probably 100 microns or less from the cells you're recording, you need very very small amounts of light. In our hands, depending on cell type, it is always sufficient to drive the neurons you're recording, but if you're looking to effect a large number of neurons that control behavior this is not the right tool in my opinion. There isn't necessarily an overt behavioral response to stimulating these neurons.
  
- 3. Can you elaborate on the method of computing the "transition probability": specifically definitions of the  $P_{causal}$ ,  $P_{fast}$  and  $\lambda_{slow}$ . What is the ground truth?**
  - a. The tissue does disperse light, obviously different wavelengths are different, the brain isn't made of water there's proteins and everything that are affecting the movement of light. The distribution of light in space and in agar, which is more similar to the brain than air, as quantified in the 2012 paper from Eran Stark and György Buzsáki that really nicely looks at the spread from small etched fibers. I think in the paper from Fan Wu, Eran Stark, Euisik and György, I don't know off the top of my head. The measurement attributed to the specifics of the LED: the driver doesn't have anything to do with it, it would be the color of the LED and the shape of the emitter. In general, the blue light won't travel as far as say red light.
  
- 4. What is the smallest # of neurons you can stimulate?**
  - a. In CA1 and the hippocampus if all the pyramidal cells are expressing channelrhodopsin, it's hard to stimulate a single cell, it's like a handful of cells. The biggest thing we need to bring that to a better number, 1 would be the best number, the biggest problem isn't with microLEDs it's with the opsins themselves. The microLEDs are great in terms of that the fact that they can light up a tiny tiny amount of tissue. The problem is that in that tissue, it's not just going to be one cell and not just the soma of one or two cells, but also the dendrites and the axons all of which express channelrhodopsin along the surface. A really big deal that we're waiting for is generating opsins that are expressed in sub compartments of the cell, such as the soma which would enable you to lower the smallest number of neurons you can stimulate.

**5. Do all neurons have the same threshold for light stimulation?**

- a. No, even within the hippocampus, interestingly the pyramidal cells, if you express channelrhodopsin in an AI32 mouse if you cross that with a cre mouse that drives the expression in interneurons or pyramidal cells they have very different thresholds for light. It turns out that the interneurons have a higher threshold, they need more light, which might sound paradoxical because they have a higher firing rate, etc. that's a repeatable effect that probably has to do with the expression of the opsin.