"Preexisting Hippocampal Network Dynamics Constrain Optogenetically Induced Place Fields" – Webinar Q&A

1. **How much/how often do you need to stimulate to induce reorganization of feedback inhibition? Is only one shot enough?**
    a. I don't really know the answer. In earlier experiments I was trying to play around with this, form one stimulation to as many as 15 but I saw so much variability in how the neurons responded I decided I had better fix one aspect of my experiment and let the brain be the variable part. So, I know that as few as 3, but in the majority of my experiments I was doing 5 stimulations.

2. **Are plateau potentials are mostly dendritic in origin?**
    a. Yes that's right, I think this is an important difference between my experiments and those that were done with the intracellular current dejection, is that I'm engaging feedback inhibition, as was a large topic of my talk, and of course a lot of this inhibition is going to come back to the dendrites. So maybe one of the differences between my plasticity induction protocol and that of Jeff McGee and others is that I'm getting massive inhibition that's coming to the dendrites, so maybe I'm having a limited amount of plateau potentials in my experiments. I don't know is the answer.

3. **Do you think that you can induce dendritic potentials with optogenetic stimulation?**
    a. I think it should be possible, if I were to do it I would recommend having very sparse labeling of the opsin expression and to not have a soma restricted version of the opsin. I did not have a soma restricted virus, it should be possible but I don't know if I was inducing plateaus in my experiments but I know I'm recruiting a huge number of interneurons and a lot of feedback inhibition that you're going to be competing with.

4. **How many neurons do you stimulate?**
    a. That's a good question, and we don't know the answer. One of the reasons that we don't know the answer has to do with the fact that when you stimulate a pyramidal cell you indirectly and synaptically stimulate the interneurons which causes inhibition so just because you don't see strong spiking doesn't mean that the light isn't actually activating the opsin. There's a classic paper by Shy Shoham (https://pubmed.ncbi.nlm.nih.gov/16550391/) talking about the dark matter in the brain, how come we don't record all of the spikes that we would expect to record from our physical modeling of voltage spread in extracellular tissue, we should be able to record 100 neurons per tetrode how come we only record 10? The real answer to how many we are able to stimulate is also restricted by the ability to record from the activity. C-fos imaging might get you part of the way there but it has it's own problems, voltage imaging will get you part of the way there but you'll still have the question of is feedback inhibition preventing your ability to see the optogenetic drive, and so I would say we're stimulating more than 10 but less than 100. That's the best answer I've got.

5. **Are you planning to use the new, low artifact uLED probes, which supposedly have stimulation artifact less than 50 microvolts peak to peak in your upcoming research on epilepsy? How might this help seizure forecasting/emulation, specifically?**
    a. Yeah this is a great question and I was just talking to some of the team at Plexon about this, so these devices will hopefully be commercially available soon and I can see this being extremely valuable because you can stimulate and record at the same time, which you can't do or maybe you can but it's very technologically difficult to do with electrical stimulation. So yes I can see some value in this, I think that future probe designs that have a larger anatomical spread of where the lights are would be particularly helpful, because here we're stimulating at a rather small volume in the brain.

6. **Do you think that stimulation during SharpWaveRipples strengthens the underlying Excitatory Inhibitory connections in a similar way? Would you think this would be why interrupting SWRs leads to less place cell stability?**
    a. Sure, I love that hypothesis I don't know if people have done the experiment where you stimulate during the ripple and show subsequent plasticity of the place fields, maybe that's in Antonio Fernandez-Ruiz paper (https://science.sciencemag.org/content/364/6445/1082.editor-summary) I don't remember. for sure there is changes in plasticity with ripple silencing, and I love this hypothesis that when you silence you're disrupting the normal kind of coupling that you'd expect during ripple, and it's important for place cells plasticity. I should say, that one of the cells that's most reliably active in the ripple is the interneuron, so if you want to have an important fire together wire together model then activity between the pyramidal cells and the interneurons during the ripples is a prime moment to have that kind of plasticity

7. **You think that is possible to stimulate LFP as well?**
    a. When I stimulate if you enough power you can induce an artificial high frequency oscillation that you can see in the LFP and I deliberately tried to titrate my stimulation so that when the animal was in the home cage I just barely got to high frequency oscillation, when the animal is running and in a theta state you can see that the overall inhibitory tone is higher, and so an equivalent amount of stimulation never induces that kind of oscillation. I think that is known from several lines of work that the threshold to spike is higher during the theta than non-theta state. If the question is whether you can induce changes in the LFP with these microLEDs stimulation the answer is yes, there are publications from Eran Stark (https://www.sciencedirect.com/science/article/pii/S0896627315009265) specifically studying that.

8. **What do you think is the source of the fluctuations in neuron-pair coupling (independent of stimulation)?**
    a. I think that one of the cases could be that you're getting inhibitory modulation of the presynaptic terminal, one of the cases could be that you're getting changes in the coupling from the spine to the dendrite, one of the cases could be changes from the dendrites to the soma. I think that all of these could be modulated by the activity that's ongoing in the local network and I think that all of these could be modulated by different neuromodulators, for instance acetylcholine. Certainly, one of the big factors that is changing which synapses need to be high and low is asleep/awake. There could be a number of neuromodulatory effects, I should say that the synapses that are rising and falling together you could have one postsynaptic cell with one synapse that goes high and one that goes low and the same with presynaptic cells, so it does look like the synapses can operate differently than the soma itself.

9. **How uLEDs are driven? Do you use a function generator or a current source? What is the best choice?**
    a. I was having this conversation with some of the people over at Plexon and I've had this conversation with Misi and Euisik a number of times. I've always driven in voltage mode, I used a CED voltage generator and what I do is I keep my voltage set to just below the forward driving voltage of the LED and if you give non square pulses and you're just below the driving voltage then you can minimize the artifact. The new generation of these microLEDs should have very very small artifacts and there I would strongly recommend going in current mode for the reasons that Misi described in his talk, the linear relationship with the amount of current you give and the amount of light output, let the brain handle the non-linearities rather than your experimental prep.

10. **How reliably can you drive a neuron from trial to trial?**
    a. We can be lulled into the sense that we have the ultimate control with our experiments we turn on the light and the brain is going to obey exactly what we do but of course if you look to see what's the firing rate of a neuron that's being driven by the light and correlate that with the rate of the spontaneous events you see a very strong relationship, a strong correlation. Embedded in one of the plots that I showed previously is, pairs of cells that have a strong synaptic coupling between the pyramidal cell and the interneuron during the spontaneous case also are strong stimulated as well. So our stimulations are engaging natural circuits, so after the first several couple of milliseconds you have all of the different recurrent inhibition that's coming in, you're also stimulating, the effect of your stimulation in kind of dependent on what's going on in the brain at the time of stimulation. I think that everyone who has stimulated the brain can see for example these differences in threshold when the animal is moving vs when the animals is still. So you can see quite a lot of variability from trial to trial and that variability is not random, it's going to depend on what the animal was doing.

11. **How long can you leave the implant in place with stable recordings / does the recording quality change over time?**
    a. I have recorded successfully for over four months. I have seen that with repetitive, thousands or maybe ten thousand stimulations something can change with the microLED so that the subsequent stimulations seem to leak current and effect recording quality. I have also left the probe in place for months with stable recordings and in some cases done thousands of stimulations and the recording and stimulation looked OK.

12. **What causes the "drift" in place field representation in the control condition?**
    a. Nobody knows. I think that it could have to do with fluctuations in phosphorylated creb it could have to do with patterns of inhibitory coupling like I showed you, I think that this is an important question for the field to answer.

13. **How do you know if cross-shank drive is synaptic or light spread?**
    a. For me the best evidence came from my cross region comparison with CA1 [where most excitatory neurons interact indirectly through inhibitory cells] vs CA3 and the dentate gyrus [which have strong excitatory to excitatory connectivity] because I did the exact same stimulation protocol but with CA3 and the dentate gyrus I always observed cross shank drive, but in CA1 I didn't. I think that in general it's tough, if you're stimulating an excitatory cell and you see spikes in any of the cells you're recording you cannot differentiate if it's synaptic or if it's direct optogenetic drive, the synapse is operating too fast and the charge time from the opsin is too variable, at least if you're recording in these same areas. The fact that I observed spatially restricted drive in CA1 and not in CA3 leads me to believe that my light is not strong enough to be inducing cross shank drive in these experiments.