"How cortical population activity is integrated for visual perception: an optogenetic approach." – Webinar Q&A

1. **It would be great to hear how you go through the dura, our lab has had some issues doing that in V1, also what is the diameter of the U-Probes you use?**
    a. We use fairly chunky U-Probes, around I think most of these ones we're using are 360 microns and that's on purpose to give it that kind of rigidity. Let's see can you guys see my video? I actually brought a handy prop, so I found this in my random collection of Halloween things but I thought it was a lot like the probe… can you see it? So, imagine this is the U-Probe. This is the part that connects to the headstages and then this is the length and imagine this little thing is the guide tube. Usually what happens is that you're advancing down the guidetube but then at some point you start seeing some buckling so what I do at this point is this. We have a NAN Microdrive system, so it clamps right underneath the head stage base. So I'll release the clamp and that corrects the alignment, but then you have a U-Probe that's sitting loose in the clamp. Next, I'll take a pair of fine tweezers or little forceps and then I'll pinch the probe just above the guide tube - not pinch hard (I wouldn't recommend this if you don't have good hands) if you have a delicate touch you can grab a bit with forceps and then I take my finger at the top and I'll guide the U-Probe by pushing down at the top and then use the forceps to pull it in. Remember that the clamp of the microdrive is still in place so that's gonna act as a stop. You'll hear a 'pop' and a rush of signal once the probe goes in. This way you have some control over how much force you're actually inserting and then because you still have the clamp there, it'll stop at the same depth and then you can re-secure the clamp and just continue. So there's a little bit of manual force, as I mentioned, that will get you through those really rubbery parts of the dura.

2. **What was the rationale for pulsing the laser and limiting the duration of the stimulation to 250 milliseconds? Had you explored more uniform and long duration laser stimulation?**
    a. Yeah, so one reason we use the pulse is that we do need a lot of light and you don't want to have to worry about heating the tissue. The other consideration is your particular opsin. Our variant of channelrhodopsin at some point it kind of inactivates so if by having the light pulsed, you give the channels some time of reconfigure themselves back to their baseline state so they're able to open again. Even with that, at 35Hz, what became clear is that each subsequent laser pulse you actually get fewer and fewer spikes each time and that seems to refresh in the intertrial interval. So you want to be careful of things like heating. You don't want too much light and you'll want to look at the actual channel kinetics of what opsin you're using.

3. **Did you evaluate the impact of the electrode implant array on the tissue?**
    a. No, so the closest thing we've done is… not really… we have done a biopsy for the GtACR2 but that was mostly to see whether or not the virus expressed but it was hard to tell actually the impact it has on tissue damage. I'm sure it's having some damage.

4. **Great talk! What role do you think synchronization between light evoked and stimulus triggered firing plays in the facilitated behavioral readout in near versus far settings?**
    a. Well, it's not quite synchronous. The laser comes on first and then the stimulus response will come on at that conduction delay, so the actual laser and the visual stimulus are synchronized but the actual response in V1 is a little bit asynchronous. I'm not sure if it's the synchrony itself that makes a difference, but I've never actually looked, it's a great question.

5. **You mentioned optoelectric artifacts during your talk, how did you ensure that the affects you saw were not due to these artifacts at least in part?**
    a. So that effect is usually in the LFPs. It's a low frequency thing, you won't see it in the spikes. Usually it gets filtered out of there. Also it's something that you want to play with at the start of your experiment once you figure out the settings to see what that threshold is, where you actually see the artifacts. So we would just titrate down the laser, the power of the laser, until we're sure we're not getting any artifacts. But either way, at this point I still wouldn't analyze the LFPs on this dataset. You may see something and then you're not sure if it's real or not.

6. **Have you tried ramping your light stimulus? We find that with square wave pulses the effect of the light diminishes, we have been ramping on over a few seconds.**
    a. No, I haven't. That's something I have been meaning to try actually mostly with one of the other opsins that sometimes produce rebound effects (at light offset). I think the ramping definitely diminishes that but no I haven't tried it yet but it's good to know it works.

7. **What are the latencies after activation of ChR2 and the inhibition opsin?**
    a. The ChR2 is actually pretty quick and it's much easier to notice the activation. When we did the measurements for the light spread, I think two to three milliseconds was considered direct even if that's a little bit permissive. For GTACR2 it's a little bit more difficult. The suppressive effect definitely seems to build up. It's a little bit slower, so it takes a longer time to notice that drop in firing rates and then the channel itself takes a long time to close so it actually stays open for hundreds of milliseconds afterwards. So as long as you know this you can kind of tailor your experiment.

8. **What do you hypothesize is the mechanism by which higher order visual areas ignore V1 activity when it comes to decision making for the behavioral perception task?**
    a. Yeah, that's a great question. I was honestly really surprised when I saw how much activation we were actually like how much signal we're injecting in there and here's my hypothesis. I think if we train the animals to detect the activation, they could learn to use that signal however because we didn't explicitly train them to be aware of the signal we're injecting I think they just focused more on the stimulus activity and the rest - the other signal that we're injecting - that was considered noise.

9. **What if one were interested in LFP?**
    a. LFP and optogenetics, you could do it maybe – the artifact is produced just when the light is turned on and off - so if you use continuous light you really should just see the artifact at the light onset and offset. You might be able to just to cut out those little portions and look at the LFP during the light is on it's really just like the transient changes or you could look at the time period before or after the light is on because there are there are network effects that happen long term or you could look at a distal site. You could just move apart the light source and the fiber optic like I showed you - 300 microns is enough.

10. **Have you tried to use tapered fibers rather than adjusting the angle of your fiber?**
    a. So, we also penetrate the dura with the fiber, so the fiber itself is it's pretty blunt at the tip but it's inside like a cannula that's beveled. So, you talk about tapered fibers kind of get more light out of them, no I think someone in the lab tried to make those once, but we have not really gone that far, we find that just like a 200 micron fiber this way seems to shine enough light for us to get our effects but yeah the tapered fibers definitely might get more light.

11. **Is V1 the only network involved in this perceptual decision?**
    a. No of course not. So the interesting thing about V1 is that you need V1 for normal vision but from what human studies have shown is that in V1, you're not really conscious of what happens in V1. But you need V1 to send visual information to higher areas that eventually become closer and more related to the actual perception. So this study relates to very early visual processing, and how that very important information in V1 gets transmitted downstream. But there's a lot more steps along the way to perception that need to be addressed.

12. **What is the power output range you use for stimulation?**
    a. I think it was it varied somewhat, I think on average we had about… we measured somewhere between 5 and 15 milliwatts per millimeter squared at the end of the fiber."

13. **Thank you for the interesting talk, what total volume of virus do you inject and have you any experience of using retrograde AAVs and if so, are there any special considerations you need to take into account when using these viruses?**
    a. I've never tried the retrograde AAVs. Those sound really cool but I have no experience with them. Most of the stuff we've done is actually antivirus mostly because it's a little bit more specific towards excitatory cell expression which is we just wanted like some part of that would have to get everything so the volume of virus we would do is five microliters per column and then it depended… what sometimes we needed to inject some more so then we do about either so each column is five times either like six or eight up to 11 other columns so quite a bit total but it was mostly just to have a large enough target site essentially because even in that four to six week period between when you inject when you come back to record - even if you do everything to make sure that grid is in the exact same spot - things still move around a bit. You're still going to be fishing a little bit, so for your injections you just want a large enough target area.

14. **Does optogenetic stimulation change the orientation tuning curve (e.g. how wide the curve is) or contrast response function (e.g. change the saturation point or steepness)?**
    a. For that particular setting we didn't actually calculate contrast response functions for the cells. We did it for the inhibitory one, as far as I can tell it seemed like the stimulus responses were fairly the same, we didn't… we were always presenting the same orientation of the cells you could probably do some kind of adaptive experiment with it but I'm not sure what the results would be.

15. **Regarding your experiments inhibiting excitatory neurons and recording from a distal site what is the hypothesis regarding the network effects that cause some neurons to decrease activity but others have different heterogeneous responses and how do these mixed changes in neural activity explain the behavioral effects?**
    a. I kind of put them through the end of that really fast, so I think what's happening is you have two columns that are side by side. There's a lot of horizontal projections that pass through layer three so I think what's happening is that you have the suppressive effects are due to mostly to just a lack of input from the load from them that usually act to… I guess they increase stimulus responses and then what happens is as you go kind of deeper in that column, so layer 2-3 is going to receive that direct input from the suppressed column as you go further down you start evolving more interneurons and then you start once you have an interneuron what kind of change is the way that cell is related to this lateral network so you might have some cells that are actually being suppressed by the local network at some contrast and other cells that are being either so the ones that actually show the facilitation. It doesn't show much response at the low contrast so it seems like at high contrast they're suppressed by that just because when we suppress them with the column measure it activates so perceptually I think what's happening is that you have some kind of heterogeneity between the two columns or between like adjacent sides and V1 so you have some that are more strongly connected I guess. The horizontal projection another ones where it's a little bit more heterogeneous with you might actually have stronger interneuron connections. We tried to figure out whether or not it was a proximity to a pinwheel center where orientation preference kind of changes more rapidly in terms of just physical topography of V1 and that didn't seem to be the case, but it could be you might need a slightly more refined measurement of this so anyway the idea is that you just have the lateral connection between the networks are slightly different and then the perceptual effect you're going to elicit is going to be a summation of those two things, so you're going to have if you have a kind of a very heterogeneous local network and you know with a uniform suppression you might not see any behavioral change whereas if you happen to be in a patch of network that's more strongly connected and you just see like a spread of inhibition predominantly then it might be easier to see that the behavioral suppression. The other thing to notice is that where we see behavioral effects there's really a low stimulus contrast so you want to have like a not subliminal but like a very hard to see stimulus and then you'll be more likely to see the impact that you're having with the epigenetic manipulations.

16. **After laser lighting did you try to distinguish a direct FR effect versus effects that would have occurred later and possibly coming from the population which was activated first (i.e., not directly due to light in the other column but from the same column)?**
    a. I'm not sure, well for the first one for the excitatory experiment we took everything whether it was directed and direct assuming that it was just the sum population activity that was actually mediating this in this oppressive experiment it was all indirect effects because if we didn't once you get away from the light you don't see any direct effects on any cell in the column you only see these funny heterogeneous effects that are only evident when there's a stimulus present so you're activating the local network. I hope that answers it follow up with if that makes sense.

17. **Did you always inject virus in a single session and was this one long anesthetized session and did you ever do repeat injections on multiple days, or would the animals develop immunity to the lentivirus making subsequent injections less effective?**
    a. So the immunity thing seems to be more of an issue with the AAVs, I don't think I've seen that with lentisvirus, we have done multiple injection levels we do it awake actually so it's not different than a recording session so we can either have the end of the test or just watch movies and then we'll do I think total in a whole day we can only do about 20 microliters at best so in order to get more if you want to do anything more than what I should like four columns in our setup we probably do multiple days but we never saw… I never saw less expression with the subsequent injections it always seems fine

18. **You used 35hz laser stimulation have you tried to match the stimulation frequency to the intrinsic gamma band recorded during the contrast discrimination task without stimulation say 40 or 50 hertz?**
    a. So the 35 hertz chosen was mostly because of this particular channelrhodopsin and it wasn't like ChETA or ChIEF, which can follow faster rates. This one, when we did some preliminary experiments, we found that 35 was about as fast as we could go without losing spikes. Essentially, it's like where the channel couldn't keep up fast enough, I would like to use some faster channels though.

19. **Thank you for the interesting talk. Are these single units? Also, what effect would you expect in the mouse visual cortex which doesn't have columnary structure ("Near" and type one).**
    a. It was a mix. We combined single unit and multi-unit. Usually what I'll do is pull out the single units just to make sure that the effect is there and that I don't mind including multi-unit as well. In the mouse - it was interesting - actually we need to find that paper it was published I think right before our Nature Communications paper came out. There was a study that did something very similar in mice where they were using a two-photon system to activate single units. They would simultaneously, well close to simultaneously, activate units that were either of a similar orientation preference or different orientation preference and they had the mouse detect whether or not they saw something. What they found was that is quite surprising. It's a similar principle in mouse, even though the architecture is different. They found that if they could simultaneously activate similarly tuned units the mouse would detect it, whereas if the same number of units were activated that had more heterogeneous tuning, the mice would not see it. So the principle is the same but the architecture is different between monkeys and mice.

20. **How long does it take you to inject one column with five microliters?**
    a. It's extremely slow the way we do it. One column will take an hour… more than an hour an hour, two hours somewhere in there. It's very slow. We also leave a lot of time in between the injections. So we'll put the needle in place, wait 30 minutes just to allow everything to settle down and then we'll inject and then we'll wait five minutes at least. I think the first time we wait even more and then we'll retract a little bit, wait for things to settle and then inject and then wait again. The idea behind going slowly is that you don't want to move everything - you don't want to drag the virus back up as you retract, so everything is extremely slow.