"Examining mesoscopic-scale networks in the primate amygdala using linear electrode arrays" – Webinar Q&A

1. **You show all this activity in response to basic sensory stimuli, but have you looked at reward related activity in the amygdala or something more explicitly tied to emotion?**
    a. Yeah, I think that's a very common question, the data I collected at the University of Arizona I wasn't explicitly looking at reward processing because it wasn't the goal of the project, however at the Oregon Health and Science University with Dr. Costa, that is one of our explicit goals. We have started to look at not just reward processing on its own but also how things like reward prediction errors are encoded within amygdala activity. It is very clear that that type of information is encoded in frequency band specific ways and I'm hoping to sort of expand, in the very near future, into looking at those more emotion-specific processes in these data.

2. **Do you see any coupling between the single unit activity and the LFP oscillations captured in the component time series?**
    a. We did start to look at spike field coherence in these data. We took a poster to SfN a couple years ago on that idea there is some very clear coupling and it seems to be particularly potent at lower frequencies. So delta and theta coupling with spiking activity was pretty prominent in these data sets. One thing that we struggled with was just getting enough neurons simultaneously recorded from the amygdala, it's just a quiescent structure, and it's hard to get a lot of neurons recorded at one time for these types of analyses. But in our upcoming experiments we're going to be using V-Probes with 32 channels, we're hoping to get a better collection of single units to try to do some more spike field analyses. It's definitely something that's on our plate.

3. **Has source separation been used to differentiate between cortical layers?**
    a. That's a more common approach, obviously cortex has a nice spatial structure in that it is layered. I don't know if people have used generalized eigendecomposition for source separation cortical layers but certainly people have used independent components analysis and other source separation techniques to look at layer specific processing. The only paper that's coming to mind is the Buzsaki paper (Senzai et al 2019, Neuron) that used ICA (independent component analysis) and they had a lot of success with differentiating activity in the LFP from the different cortical layers. That's a pretty straightforward and common approach for analyzing activity in cortical structures.

4. **How do you determine that six millimeters would be enough to span the amygdala was the 7T scan necessary for that and did you experience variability in size between animals?**
    a. There's definitely variability between animals to a degree but in general I've worked with adult Rhesus males for most of my career and for the most part it's about six to eight millimeters in the dorsal ventral expanse as you move through the amygdala. We would always do a structural scan (T2 weighted, 0.5 mm isotropic voxels) on our animals before we would do any type of recording to make sure that everything is intact and healthy before we go in. We can use those structural scans to get an estimate of the size of the amygdala, but I would certainly say while you need to do those ahead of time you don't necessarily need to do a 7T scan after the fact (i.e., ex vivo) to get that sort of readout of the spatial structure of the amygdala.

5. **Do you have recommendations about which drives to use in combination with the V-Probes to get clean LFP recordings?**
    a. So obviously it's going to be dependent on your lab, when I was in graduate school I used motorized electrode manipulators or MEMs from Thomas Recording, they're a company out of Germany. I really liked using those micro drive systems because they gave you a very precise readout on the micron level of where the estimated position of your electrode tip would be. Using pencil drives with these arrays is great because you can put down you can put pencil drives very close to one another and you can potentially get down multiple arrays in a small area but I don't think you necessarily have to go with any one particular drive system. I think where I am now (Oregon Health and Science University) we're gonna be using a NAN system to deliver V-probes but I think you just have to sort of look at your recording setup and try to figure out what's gonna work best for you. And I should maybe put in one little plug, Thomas Recording did work with us while I was doing my PhD to create drive systems that allowed for you to basically attach multiple probes to one another on the same drive so you could put them all at the same dorsoventral depth which was super useful and it basically fit into our already existing grid structure. So if I was going to have to make a recommendation I would go with what I know which is Thomas Recording, but I don't think that's a requirement for getting these data.

6. **Can you describe more about your methods of implantation? Do you pierce the dura?**
    a. So, we use cannulae to pierce the dura. We keep our probes contained within a 23 gauge cannula so those were lowered down acutely and those cannula were used to puncture the dura. We didn't do like a dura slit or anything like that in our recordings. I was using a V-Probe and not an S-Probe. So the V-Probes don't have the steel tip, I didn't feel comfortable trying to push those through the dura. I will say there have been times where I've mistakenly not lowered my cannula low enough and tried to push a V-Probe through dura and it did not work, they bend, and thankfully you're usually able to catch that before any damage is done but you do have to get those delivered through the dura and in some manner or else you're gonna end up breaking your probes.

7. **Could you describe how you go about targeting the amygdala on a daily basis also since you have to go through so much tissue do you notice damage from repeated penetrations.**
    a. With the arrays we have because they span so much of the dorsal ventral axis, I generally just try to pick a depth that's going to put my ventral contacts near the base of the basal nucleus and then I just move around medial, lateral, anterior, posterior, to try to not go to the same areas on consecutive days. I think David mentioned in the last talk that you do get tissue fatigue if you try to go to the same spot, especially at the single unit level you tend to not get as good of isolation of neurons on those subsequent days. In the past what I basically tried to do is use a recording grid and I just tried to march through the points in that grid such that I was always staying a couple millimeters away from my previous recording site. However, in my recordings that I'm about to start in the next week or two here in Oregon, our initial plan is to put down at least four 32 channel V-Probes into the amygdala and with that I'm hoping that we basically only have to do one or two recordings in order to get enough data to achieve the goals we have. So for that I'm probably only going to have two configurations planned and if we don't have enough data after that recording we'll probably let the tissue settle for, I don't know maybe a week or two, and then try to go back. But I think the goal with using more of these linear electrode arrays is to not have to go back and record session after session. It's going to be get in, perform three or four total recording sessions with the animals get enough data, and then be able to seal them up and let them go back to, you know, living their monkey lives. One thing to note: I did dozens of recordings per animal during my PhD work. In one animal, damage was so minimal it was hard to visualize even in the histology. But in another animal I worked with, some degradation of the striatum and tissue above the amygdala did occur. The main area I saw damage was in the cortex where our cannula penetrated. The probes themselves are pretty low impact, in my experience

8. **With the high cellular density of the amygdala have you ever tried using tetrode probes for your recordings and have you ever used chronic recordings in the amygdala?**
    a. I have not used tetrodes or chronic recordings. I do know of groups that do. With primates, my personal opinion is that you don't necessarily need tetrodes, with the 16 channel V-Probes that we use with 400 micron spacing I very rarely saw waveforms from single units being picked up across multiple channels. I generally didn't have too much problem isolating individual units off of those linear electrode arrays, I mean occasionally it was a little bit tricky, but I don't think tetrodes are a necessity in the primate amygdala, like they are maybe when you're recording from hippocampus or you know maybe a more dense cortical structure. For chronic arrays, again, I've never done that but I know there are there are plenty of groups that have. The one that pops to mind is David Leopold's group and his bundle of electrode fibers that he uses which I think are wonderful. You get great cell stability over time but the problem with his recording technique is that it can be difficult for you to know exactly the position of each of your electrodes within the amygdala is and for me that spatial configuration is really important. I really want to know about nucleus anchored processing loops so if I could have like an array (i.e. the Utah array with multiple shanks) delivered to the amygdala with a set spatial structure where I could be confident in the position of my electrodes I would definitely go for that but right now I don't think that technology is widely available. I know of a group that's trying to get there but it's certainly not something that's accessible, at least for me right now.

9. **Why did you use 400 micron spacing?**
    a. So the most honest answer here is that 400 micron spacing gave us a solid coverage of the amygdala so it gives us most of the dorsal ventral expanse covered without wasting too many contacts potentially being outside the amygdala. But there's a lot of I think benefits to using that 400 micron spacing (with respect to LFP recordings). One is you won't pick up the same unit across multiple contacts so cell sorting is pretty easy, you don't have to worry about duplicating cells across channels. 400 microns means that the LFP that you're picking up across those different channels tends to be become fairly unique as you get across a couple of electrodes. So you don't have the exact same signal being picked up on every contact. It's not so much of an issue when you start to apply source separation techniques to your data because you can isolate the statistically independent signals from these different networks. When we started these experiments, we didn't exactly know how to do that, though. That's sort of the reason why we went with 400 microns, it is just to get good coverage of the amygdala. You could go smaller if you had interest in more local networks. We've even talked a little bit about going larger and trying to have coverage of structures dorsal to the amygdala, things like the nucleus basalis and the amygdala at the same time. It's sort of looking up to what you want to do in your experiments as to how you design your probe.

10. **You mentioned you didn't see differences between each area of the amygdala when looking at single unit recordings, are the neurons in these in these regions similar in type and function? If not can you elaborate on why you didn't see this?**
    a. The amygdala is where I've spent a lot of my time so I don't know how I can necessarily compare to other brain regions super well but there are a lot of heterogeneous neurons in the amygdala. I think that the reason why we didn't see a whole lot of spatial structure to the signals at the single unit level is just that it's very difficult to get a sampling that's going to be sufficient to sort of get to that level of information. We recorded a couple hundred neurons from a couple different animals but the primate amygdala has six million neurons in it, it's got as I said there's 15 compartments in the Paxinos atlas. Fifteen different nuclei that all have different cytoarchitectural features. Getting enough neurons from each nucleus to be able to look at nucleus specific processing at the single unit level I think would just be a very difficult endeavor. Now if we spent a lot more time with high density arrays I think we could get there and I certainly think that's worth pursuing but I think the reason why we didn't see it in those particular data was just that we couldn't get a large enough sample. I think that had we had access to maybe the 64 channel probes, had we been able to put down multiple probes per recording and had we been able to isolate cells more in the thousands range, maybe we start to see that spatial structure arise (at the single unit level) but that's something that's going to have to be addressed I think going forward technologically in order for us to get to those types of questions at the single unit level.

11. **I have 16 electrodes in one area and they show different LFP power, how would you do LFP analysis? Do I have to average of all of the electrodes or do I have to pick one electrode?**
    a. I use a common average reference so I average the common signal across all contacts for my data which is something you can do with the Plexon electrode arrays. In fact there's different referencing configurations you can put in to either reference to the shank of the electrode or you can set yourself up for common average referencing (or referencing to a single channel even). You have a little bit of play from Plexon's technology which is great but I use a common average reference when I go to do my analyses because different contacts are picking up some of the same signals. Therefore, subtracting out the common signals across all channels helps get rid of these global signals that may be generated by something like EMG from the muscles on the head or EOG activity from the eyes. Again, I'm going to always go to source separation techniques. For those I can't necessarily tell you what is right for your data. If you are interested in learning more about them I would again highly recommend checking out Mike Cohen's YouTube series, or he has a textbook on analyzing neural time series data and he's also got a linear algebra textbook for source separation in neuroscience. All of those resources I think would be great in sort of pointing you in the right direction but without knowing a lot about your experiments I couldn't really tell you the best approach for you specifically."