In the first chapter I presented evidence of single neurons in the human hippocampus that reinstate their (temporal) firing rate during the retrieval of specific episodes. These Episode Specific Neurons (ESNs) are distinct from neurons that are tuned to specific concepts (Concept Neurons) or reoccurring time points (Time Cells). There is preliminary evidence that these ESNs do not exist in the parahippocampus, although our coverage in that area is worse than in the hippocampus, and that ESNs are likely excitatory pyramidal neurons.

In chapter 2 we extended these findings to the HF band in the LFP. Although no consensus has been reached ikn the literature it is generally agreed upon that an increase in HFP reflects an increase in spontaneous neural firing. In parallel to our earlier findings we demonstrated that power in the high frequency band (40-200 Hz) is reinstated for specific episodes in a significant number of microwires. This finding was limited to later remembered episodes and did not crystalize for later forgotten episodes. Although we did find high frequency power (HFP) modulations akin to the firing rate increases in Concept Neurons, these HFP changes were not responsible for the HFP memory reinstatements.

Unexpectedly, the relative power increases in reinstated episodes extended past our frequency range of interest (10hz? For encoding and 15hz? For retrieval). Future studies should differentiate between a power offset, a 1/f shift and oscillatory drivers.

Based on influential theoretical work by Hasselmo and colleagues in the third chapter we expected single neurons and ESNs to lock onto different phases of theta. Recent work has shown that hippocampal theta in humans is divided between a slow (2-5 Hz) and fast (5-9 Hz) theta oscillation. Contrary to our hypothesis we did not find a significant phase preference during encoding or retrieval of episodic memories consistently over two independent datasets. We also di not find evidence for a theta phase offset between encoding and retrieval. This is in line with previous research showing that although neurons lock to a preferred phase of the ongoing theta oscillation this phase is not shared across neurons. Indeed, many neurons might not be involved in processing a given memory providing a possible reason for our findings. However, this does not hold true for ESNs which by definition code a specific memory. The absence of a theta phase effect in this case possibly lies within the low number of ESNs leading to insufficient power to detect an effect. [not sufficient periodic theta?].

To conclude, we found a single neuron basis of memory processing and extended/embedded these findings to activity in a greater population of neurons reflected by the local field potential. While there remain many exciting open questions we hope to have laid a foundation for future work.

Future studies

Although our research that culminated in compelling evidence for esns was inspired by what Teyler and DiScenna called *Index Neurons*, we did not call them such. This is because there are features ascribed to Index Neurons that we cannot test using the two available datasets.

Upon presentation of a partial input present at memory encoding the index neuron assembly in CA3 is pattern completed. As we lack the sufficient coverage to record multiple neurons of one assembly that codes an episode, we can not investigate this. Relatedly, pattern separation should allow the distinction of highly similar, but different episodes through allocation to different index neuron assemblies. We are unable to verify this using the current experiments because the images used in each episode do not overlap. If Index Neurons allow memory reinstatement they should reinstate their firing pattern on all subsequent retrievals (although some variance due to memory consolidation is to be expected). Patients in our studies retrieved every episode only once. We are currently running an experiments where each episode is retrieved multiple times, but will not have a complete dataset in the foreseeable future.

A central part of the indexing theory is that ongoing cortical activation is bound by neurons in the hippocampus which project back and reactivate the initial cortical pattern during successful retrieval. There are several hurdles in showing this empirically. One is a low spatial coverage of implanted electrodes that is on top of that different for each patient (why is that ba d xx). The number of ESNs per patient is low. It is not clear at which timepoint memories are reinstated in the hippocampus based on the firing of individual ESNs. These concerns should not deter the curious reader, but merely caution to the difficulty of the task.

Only future studies using other experimental designs and/or newer hardware will be able to ascertain whether ESNs are Index Neurons. Until then we have to be satisfied to see a reinstatement of neural firing as an indicator of memory processing.

Another interesting question is the stability of ESNs over time. Does the index reliably reinstate a memory a day after memory encoding? What about a week? Note, that it is a separate question as to whether the hippocampus in general stays involved in older memories (multiple trace xx) or not (systems consolidation/two stage xx). This is because it is conceivable that the initial memory trace is transformed during the consolidation period. In light of this, being able to record more single neurons or even multiple ESNs that reinstate the same episode would be especially insightful. Otherwise, one might falsely believe that a memory trace is being erased from the hippocampus when in reality just a part of the initial neuron assembly coding for an episode has been pruned off. This is where our finding that HFP is being reinstated / ESNs are reflected in HFP comes in handy [expand, make more formal].

A related question is if the index is being systematically reactivated during the consolidation period, especially longer periods. Recording assemblies would possibly allow identifying points of reinstatement by making it possible to differentiate background firing from memory reinstatement [can we look at spike locked HFP?? Possibly get different results if you have ESW that reinstate the same episode as ESN; maybe only works then. Look at bimodal or lopsided distribution]. Sleep should be a major time period of interest when looking at memory consolidation (Kolibius et al., 2021, xx others; generally expand on this].

As I am writing this Neuralynx (xx) is seeking CE and FDA approval for microwire stimulation in patients (personal communication). If successful, microwire stimulation could provide causal evidence for an ESN based memory code. If ESNs are allocated based on excitability stimulating the neurons in the vicinity of a microwire should increase the probability they are allocated to an episode. This would shed light on a mechanism of ESN allocation predicted by animal work and also increase the yield of ESNs per patient. Using this method one could test the hypothesis that CN develop from ESNs by stimulating on a microwire during multiple episodes that shara a common element (e.g., Jennifer Aniston in Pisa, Jennifer Aniston in Paris, …). If the stimulation causes coallocation of neurons to these episodes it is conceivable that some of the “tagged” neurons exhibit neural firing akin to Concept Neurons tuned to Jennifer Aniston (as she is the common element in these episodes).

An open question remains how CNs initially develop their tuning. One possibility is that over repeated reconsolidation CNs evolve from ESNs. Imagine you meet your best friend in a coffee shop. This coffee shop episode will initially be represented by an assembly of ESNs. A few days later you meet with the same friend in a park and you remember the last time you met in the coffee shop. This reactivates the ESNs coding for the coffee shop episode. Engram literature suggests that recently active and more excitable neurons are preferentially bound to a new episode (Josselyn and Frankland, 2018). This makes it likely that some of the ESNs that coded the coffee episode now also code the park episode. The shared content between those two episodes is your best friend. It is conceivable that over many such similar episodes a proportion of the ESNs that initially coded the coffee shop episode would become "semanticized" i.e., develop a tuning for your best friend. A Concept Neuron is born.

In this way ESNs can be likened to variables in a computer program to which arbitrary information is bound. In the case of episodic memory, this arbitrary information would be the complete set of features that make up an episode.

Overlapping content? In context of pattern separation.

DISCUSSION NEURAPIXELS

Not in all cases can this difference in neuron yield be made up by recording more participants. In the context of this paper, recording hundreds of neurons in one patient would enable the analysis of between cell interactions, for example the detection of assemblies of Episode Specific Neurons. Something that is not feasable when recording few neurons.

Beyond this, between area interactions could be investigated (Durand et al., 2022 Nature Protocols). One concrete example would be a reinstatement of cortical representation driven by hippocampal ESNs. During encoding one would assume that the cortex is driving the hippocampal activity, while during retrieval the cortex lags behind the hippocampus (some study by maria wimber).

Discussion

Discuss information flow between hippocampus and cortex

You could do granger causality with the raw data

alternatively granger causality on the instantaneous/time-resolved output of a classifier (with the focus would be slightly different instead of just looking at the raw voltage predictions from hippocampus to cortex or cortex to hippocampus, depending whether its during encoding or during retrieval, you'd look at evidence for certain content and during encoding that should occur in the cortex earlier and in the hippocampus later and vv during retrieval. Another way would be to look at the classifier output in the cortex as a function of the instantaneous firing rate of ESN after binding

same for high frequency power. look at hfp in a time-resolved fashion. you can look at HFA&Theta coupling (there is a bunch of literature on gamma&theta coupling although HFA is not gamma; HFA is likely aperiodic, mention the correlation between HFA and spikes but spike&theta coupling if there is something interesting)

We always use the same cue for one episodie, if we want to look at pattern completionin more detail we should use varying cues. Still the same index should be reinstated. Maybe having neurapixels probes and/or more ESNs from one ESN assembly recorded might make that more interesting as well. Like the order in which they are reinstated

Once you can create ESN on demand you can check whether their firing rate follow the probability of the presentation of stimuli. A la polish paper with CN in a pyramid that I sent to Turk-Browne

Include Ison in the discussion about engram allocation, because it lines up (then also introduce quickly in intro)

Idea is this experiment:

A+B = C

A+D = E

A+F = G and stimulate each time. Do CN tuned to D fire to A and E?

Do they fire to B&C and F&G? IF so, in line with repetitions? Let's say A+B=C is more common then another trl is tun/reflected in re-tun?

Do we get a stimulus A CN? Does this change throughout the day/nights/experiment?

Do we have a ESN first? So for A+B=C?

Without stimulation (?) a+b=c

a+d=e

ESN for one overlap?

How are ESNs over repetitions? How does tuning response change? Repetition on next day as well? Pattern completion?

More to 3B

Assuming that the high frequency activity in the LFP in each high impedance microwire is independent, but in reality they are referenced using the same/a shared low impedance microwire. This is a problem depending on how local the high frequency component is (if it is only picked up by the low impedance microwire then they are all reflecting the same signal + noise; this is fine because the same is true for the 2nd order oscillation; if they reflect very local activity they might differ or form clusters of neurons that all have the same spatial orientation; we do not know how they relatively move during implantation). Hinges upon how local the high imedance and how local the low impedance wire records. HAven't found anything on behnke fried, only in the buzsaki paper on 100ym spikes and 250ym lfp but that was for grid electrodes.

gaussian for convolving is a problem for spikes~HFP. The other problem might be how I shuffle for the second order permutation. I do zscore but maybe the permuted correlation is low if I shuffle HFA of one microwire with a low firing neuron on another microwire. This should not be a problem if I shuffle episodes where I compare LFP and spikes of the same microwire.

Variance explained is only 1% (r=0.11²). Add we assume that the microwire we record the spikes on best reflects HFA/neuron input. So maybe in the future compare with other microwires on the same bundle. Also look at different frequencies. Future studies should look at individual frequencies between 40 to 200hz. Extend range a bit as well. Do something as a spike locked LFP? I did a time frequency plot but it wasn't anything nice.

correlate each single neuron firing with every frequency in HFP and also every microwire on that bundle. Only 1% variance explained. More neurons with neurapixels would allow us to record more neurons and look at synchrony as another driver of HFP. Also would be able to form clusters of brain areas that show a different firing~HFP relationshop (as e.g. different layers show a different relationship)

There is a lot of variability in the HFA range in the literature!