In the first chapter, we presented evidence of single neurons in the human hippocampus that reinstate their (temporal) firing rate during the retrieval of specific episodes. These neurons, referred to as Episode Specific Neurons (ESNs), are distinct from neurons that are tuned to specific concepts (Concept Neurons) or reoccurring time points (Time Cells). Preliminary evidence suggests 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 high frequency band in the local field potential. Although no consensus has been reached in the literature yet, it is generally agreed upon that an increase in high frequency power reflects an increase in neural firing (xx). In line with this, we found a significant, albeit low, correlation between single neuron firing and high frequency power. In parallel to our earlier findings, we demonstrated that power in the high frequency band (40-200 Hz) was reinstated for particular episodes in a significant number of microwires. This finding was limited to later remembered episodes and did not emerge for later forgotten episodes. Although we did find stimulus dependent 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 in ESWs. Unexpectedly, the relative power increases in reinstated episodes extended past our frequency range of interest (until ~10 Hz during memory encoding and ~15 Hz during memory retrieval). Future studies should differentiate whether this finding is driven by a power offset, an aperiodic 1/f shift, or oscillatory components.

Ample research points towards a central role of theta in episodic memory processing. Recent work has shown that hippocampal theta in humans is divided between a slow (2-5 Hz) and fast (5-9 Hz) theta oscillation.However, the exact role of theta remains elusive. Herweg and colleagues (xx) proposed that successful memory is reflected in a power shift towards higher frequencies and a circumscribed narrow-band periodic theta increase. In the third chapter we tested this hypothesis in two intracranial datasets but did not find consistent evidence to support it. Based on influential theoretical work by Hasselmo and colleagues, we expected single neurons and ESNs to lock onto different phases of theta. Contrary to our hypothesis, we were unable to identify a consistent phase preference during encoding or retrieval of episodic memories across two independent datasets. We also did not find evidence for a theta phase offset between encoding and retrieval. Indeed, many neurons might not be involved in processing a given memory, which provides a possible reason for our findings. However, this does not apply to ESNs which, by definition, code a specific memory. The absence of a theta phase effect in this case may be attributed to the low number of ESNs leading to insufficient power to detect an effect.Although a complete absence of theta phase preference goes against previous findings, neurons reportedly lock to a large range of theta phases (Jacobs et al., 2007).

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

ESNs are maybe Index Neurons

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. In both of our experiments, we always use the same memory cue for encoding and retrieval. Nevertheless, we predict that using varying memory cues from the same memory should reinstate the same ESNs. Having access to multiple neurons that are allocated to the same episode would create the opportunity to ask more nuanced questions. If a cue does not initiate memory retrieval, do some neurons reinstate their firing activity, but the activation is not sufficient to pattern complete the entire assembly? Is there a relationship between strength of assembly reinstatement and detail of episodic retrieval? Is there a specific reactivation order based on the memory cue, which would indicate that within an assembly, individual neurons are responsible for specific features within the episode. It is conceivable that depending on the hippocampal subfield these answers differ. For example, reactivation in the DG might be all-or-nothing (xx) while the auto associative structure of the CA3 (xx) allows for more graded reinstatement. As we lack sufficient coverage to record multiple neurons of one assembly that codes an episode, we cannot currently investigate these questions.

Conversely, pattern separation should allow the distinction of highly similar, but different episodes by assigning them to different Index Neuron ensembles. We are unable to verify this using the current experiments because the images used in each episode do not overlap.

Another important question is the stability of the ESN code over time and multiple retrievals. We expect ESNs to generally reinstate their firing pattern on all subsequent retrievals. However, it is possible that some ESNs drop out of the assembly that is allocated to a given episode, which would lead to some variance. Patients in our studies retrieved every episode only once, so we cannot investigate this question. We are currently running an experiment where each episode is retrieved multiple times but will not have a complete dataset in the foreseeable future.

An interesting related endeavour is exploring the stability of ESNs over time. Are memory traces (as evidenced by ESN firing and HFP increases) systematically reactivated during the periods of (extended) consolidation? In this context, sleep is of particular interest as numerous studies suggest it has a role in memory consolidation by reactivating previous experiences (Schreiner et al., 2021 10.1038/s41467-021-23520-2; Kolibius et al., 2021; Born et al., doi.org/10.1177/1073858406292647). Do ESNs reliably reinstate a memory days and weeks after memory encoding? Note, that it is a separate question 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 original memory trace is transformed during the consolidation period between encoding and retrieval, meaning the initially allocated neurons have been replaced by other neurons or pruned. It is easy in this case to erroneously infer that the hippocampus becomes redundant in retrieving a distant episodic memory when in reality the hippocampal memory trace persists in an altered form (i.e., consisting of fewer or other neurons). In light of this, being able to record more single neurons or even multiple ESNs that reinstate the same episode would be especially insightful. In absence of this possibility our findings that episode reinstatement concurs with HFP increases could be used to investigate the stability of a memory code and the continuous involvement of the hippocampus in episodic memories. As a proxy of much broader neural firing HFP is likely robust to a drop out of some of the initially allocated neurons.

Information flow between hippocampus and cortex

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 as not all patients have electrodes in the same part of the hippocampus or cortex. Apart from that, the number of ESNs per patient is low. These concerns should not deter the curious reader, but merely caution to the difficulty of the task.

A persisting problem has been discerning periods of memory reinstatement from background activity. One approach to this problem might be combining high frequency activity with concurrent ESN firing, which could serve as a more accurate indicator of memory reinstatement. A second approach is to correlate the instantaneous firing rate of ESNs or HFP with the output of a classifier (e.g., a linear discriminant analysis), which represents a retrieved memory in the cortex. This is preferable to computing the classifier evidence at each individual spike because this makes it difficult to compare ESNs with vastly different firing rates and also introduces problems during spike bursts (effectively smoothing of the classifier output). A larger increase in instantaneous firing rate would also be easier to discern from baseline firing using a thresholding procedure and could be used as a marker for memory reinstatement. A third way to detect memory reinstatement would be to apply a Granger causality test on shorter data segments during the retrieval phase, when information is thought to flow from the hippocampus to the cortex (and vice versa during encoding; xx). Segments with a high Granger causality (i.e., where activity in one area predicts the activity in the other) are likely timepoints at which memory retrieval occurs. A fourth way would be to identify memory reinstatement in the cortex through a classifier and reverse engineer the hippocampal activity pattern that induced it (e.g., looking at the neural activity one second prior).

Finally, a fifth way would be applying a classifier to the hippocampal and cortical recordings separately and cross-correlate the two outputs or apply a Granger causality test. In this case one would not focus on the transfer of the signal as raw data, but rather the transfer of evidence for content that represents that memory. To complicate things further one may wish to use the instantaneous firing rate and/or data within the high frequency band instead of the unfiltered raw micro-/macrowire recordings as inputs to these analyses. To conclude, only future studies using other experimental designs and/or more advanced hardware will be able to ascertain whether ESNs are indeed Index Neurons. Until then, we must be satisfied to see a reinstatement of neural firing as an indicator of memory processing.

On the origin of Concept Neurons

Recent times have seen an explosion in neuron types. We find cells that code for spatial locations, such as place cells (xx) or grid cells (xx). More recently head direction cells and anker cells have been identified. In humans, neurons that code for specific concepts, so called Concept Neurons have been found consistently.Recent additions include novelty cells and familiarity cells (or neurons?) as well border and event cells (xx). In this work we introduced Episode Specific Neurons and added to this veritable embarras de richesse. We do not believe that each of these neurons represent a physiologically distinct neuron type that earns it's label through a separate coding mechanism. We would like to therefore muse on the open question how CN initially develop their tuning. 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 & Frankland, 2018, Josselyn & Tonegawa xx). This makes it likely that some of the ESNs that coded the coffee episode now also code the park episode. The common element between those two episodes is your best friend. It is conceivable that over many such 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.

More advanced electrodes

The yield of neurons using the currently available electrodes is about a dozen per microwire bundle. In comparison, electrodes developed more recently yield hundreds of well localizied neurons. Virtually all analyses within described in this thesis would benefit from more neurons. We have mentioned this throughout the manuscript when appropriate. Not in all cases can this difference in neuron yield be made up by recording more participants. For instance, recording hundreds of neurons in one patient would enable the analysis of between-cell interactions, such as the detection of assemblies of Episode Specific Neurons. Something that is not feasible 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 neurons that represent a memory and their interaction with hippocampal ESNs. During encoding one would assume that the cortical neurons drive ESNs, while during retrieval the cortex lags behind the hippocampus (Linde-Domingo et al., 2019; doi.org/10.1038/s41467-018-08080-2).

Microwire stimulation

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, as predicted by experiments in rodents, stimulating the neurons in the vicinity of a microwire should increase the probability that they are allocated to an episode. In other words, through electric stimulation hippocampal neurons may be galvanized - at the push of a button - into coding for a particular episode. Apart from shedding light on the mechanism by which ESNs are allocation this would also greatly 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 share a common element (e.g., Jennifer Aniston in Pisa, Jennifer Aniston in Paris, …). If the stimulation causes neurons to be coallocated 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). Likewise reactivating the previously assigned neurons through electrical stimulation should increase the probability the memory is retrieved or even induce memory retrieval in the absence of a retrieval cue. Conversely, depending on the effect of the stimulation protocol, memory retrieval may be prohibited.

Translational applications

We generate and retrieve episodic memories constantly and without any effort. So it comes as no surprise that we usually don’t think about this marvellous ability. It is thus so much more painful when memories cause problems. Alzheimer's disease is a progressive degenerative disorder that causes neurons to atrophy resulting in one of the hallmark symptoms of Alzheimer's - memory loss (xx). It is the most common form of dementia was the 7th leading cause of death in the USA in 2020 (Murphy SL, Kochanek KD, Xu JQ, Arias E. Mortality in the United States, 2020. NCHS Data Brief, no 427. Hyattsville, MD: National Center for Health Statistics. 2021. 10.15620/cdc:112079). Worldwide around 55 million people have dementia, a number which is expected to rise to 78 million by 2030 (WHO: https://www.who.int/news-room/fact-sheets/detail/dementia). I am hopeful that our research can contribute to alleviate some of the burden that comes with Alzheimer's although a cure requires a more targeted understanding of the disease. However, it might prove fruitful to electrically stimulate parts of the hippocampus during the early phases of the disease. This could help with the recruitment of ESNs to new memories or reinstate existing memories and thereby temporarily increase their quality of life.

Another disorder characterized by dysfunctional memories is the Post Traumatic Stress Disorder (PTSD). Its symptoms include vivid intrusive memories or flashbacks that can be triggered cues reminiscent of the traumatising event. Re-experiencing the traumatic memory can cause intense physical and strong emotional reactions (ICD-11). Although PTSD patients often suffer from deficits in declarative memory (10.31887/DCNS.2011.13.2/ksamuelson), a core issue is the pathological remembering of a disturbing event. Studies in rodents suggest that the size of the hippocampal engram does not increase with the intensity of the memory (xx). This raises the question whether it may be possible to interact with the hippocampal memory trace that represents a traumatic experience. Is it possible to downregulate the engram on demand, or even ablate it? The latter raises an important ethical concern - does erasing an entire experience also abolish the consequences of the experience or does it simply get rid of the conscious perception leaving the vicseral reaction intact? Of course, the idea of simply "switching off" a memory as a treatment simplifies the underlying syndrome of PTSD. Nonetheless, the idea is that through basic research such as the work presented here we gain a better understanding of how memories are processed in humans and that this insight helps translational applications further down the road.