Introduction

In the previous two chapters we investigated the formation and retrieval of episodic memories on the level of single neurons in the human hippocampus. We provided compelling evidence in the second chapter that this episode specific code - expressed through individual assemblies of neurons (ESNs) - is separate from Concept Neurons. In this chapter we will delve into the neurophysiological substrates of memory processing that is one level above that: the local field potential (LFP). The LFP represents the synchronous firing activity of hundreds of thousands of neurons (xx) in an area around 250 µm from the recording electrode (Katzner, Nauhaus, Benucci, Bonin, Ringach et al., 2009, Neuron; Xing, Yeh, & Shapley, 2009, J Neurosc). In contrast, because the amplitude of single neurons rapidly declines with an increasing distance to the neuron soma, we cannot reliably record single neuron activity further than 100 μm away with current methods (doi.org/10.1529/biophysj.107.111179; doi.org/10.1038/nn1233). We will probe the role of two prominent frequencies in the hippocampus: the theta frequency (2-9 Hz) and activity in the high frequency band (70-150 Hz).

High frequency band power increases in the range of 40 Hz to 200 Hz have been used as a proxy of local spiking synchrony and as such provides an important link between higher level EEG and lower level spiking activity (doi.org/10.1038/nrn3241). Most of the literature examining the relation of spiking activity and LFP is based on studies in monkeys in early sensory cortical areas that have a topographic structure (10.1523/JNEUROSCI.2848-08.2008 (likely due to synchronisation), 10.1371/journal.pbio.1000610, 10.1016/j.neuron.2009.08.016, 10.1126/sciadv.abb0977, doi.org/10.1038/nrn3241), but some evidence has been reported in humans (doi.org/10.1371/journal.pcbi.1000609, doi.org/10.1523/JNEUROSCI.2041-09.2009, doi.org/10.1093/brain/awu149 (during memory encoding and retrieval in the hippocampus and visual pathway), doi.org/10.1016/j.cub.2007.06.066). Although neighbouring neurons in the hippocampus are not structured topographically and often represent very different concepts (rodrigo xx) there is some evidence that the HFA-spiking relationship remains intact (doi.org/10.1093/brain/awu149, doi.org/10.1523/JNEUROSCI.2041-09.2009).

It is unclear if enough neurons are part of one assembly of ESN (see chapter 2 & 3) to increase power in the HFB, if these neurons are close enough in space and fire in synchrony. As a starting point, Ruetishauser and collegues reported that roughly 20% of all neurons in the hippocampus and amygdala responded to novel stimuli, which should be enough to elicit HFA. However, the authors do not report whether these neurons respond to specific new episodes or new episodes in general and how many of them reinstate their firing rate during retrieval. Concept Neurons on the other hand have been suggested to consist of an assembly of roughly xx single neurons (Rodrigo), which is likely not enough to impact HFB power.

We postulated a reinstatement of oscillatory power in the high frequency band from encoding of specific trials to their reinstatement during an episodic memory task. As Concept Neurons are thought to be part of smaller assemblies of about 160 neurons (xx) we expected not to find changes in high frequency power induced by specific concepts.

Research in the role of theta oscillations on learning on memory go back to the late 70s (Winson, 1978, Berry and Thompson, 1978). Winson (1978 xx) showed that lesioning the medium spetum caused impaired spatial memory along with a reduced hippocampal theta rythm. In line with this, a higher theta power in rabbits was associated with augmented learning (Berry and Thompson, 1978)

More recent findings in humans demonstrated that later recalled items are associated with a higher theta power in comparison to later forgotten items (Lega, Jacobs, Kahana, Hippocampus, 2012; Staudigl & Hanslmayr curr biol 2013; Kahana et al., nature 1999 <- all from terwal).

Another piece of evidence for the importance of theta in the memory process comes from ter Wal and collegues who showed that the behavioral response time during memory retrieval is modulated by a theta rythm (ter Wal et al., 2021 nat coms xx).

The formation of episodic memories requires the integration of multiple different elements (Tulving xx) that are represented in different modaltities across the cortex (check out Konkel & Cohen 2009 and wallenstein, Eichenbaum & Hasselmo 1998)).

These multi-modal elements are thought to bind together by long-term potentiation of synaptic connections - a process which is sensitive to the exact timing of neural firing [Markram Lübke, Frotscher, & sakmann, 1997 xx]. In humans, this timing is suggested to depend on hippocampal theta activity (Clouter, 2017 xx, check out hanslmayr staresina bowmann 2016 and the staresina wimber paper).

A central requirement of the hippocampus is the ability to encode new information without interfering with related previous experiences. Hasselmo and collegues developed a computational model that solves this conundrum by moving encoding and retrieval processes to opposing phases in the theta rhythm (xx, also shapiro turk browne botvinick nroman 2017?). Empirical support for this 180° shift between memory encoding and retrieval has been recently found by (Kerrén et al., 2018, current biology, xx).

This same mechanism of separating conflicting information in different theta phases has been shown in a proactive interference task. Here, after several repetitions the competing association occured in a separate phase of a 3 Hz theta oscilllation compared to the target association making it less likely to be retrieved (?). This phase offset effect was more pronounced when interference was behaviourally lower (Kerrén et al., biorxiv).

spike theta locking: Jacobs, kahana ekstrom fried, 2007 j neurosc & ruetishauser nature 2010

We therefore expected neurons in the hippocampus to fire at distinct and separate theta phases during encoding and retrieval of episodic memories.

Activity in the theta band seems to enable the coordination of inter-areal synchrony supporting information flow between distant brain areas (xx) and to segment or chunk the ongoing brain signal into blocks of information (expand). Recent findings suggest that there are two distinct theta rhythms governing the human hippocampus: a slow (2-5 Hz) and a fast (5-9 Hz) oscillation.   
In their influential work Hasselmo and colleagues propose that the theta phase separates states of memory encoding and states of retrieval by 180° with the aim to minimize interference between the ongoing experience and previous memories (10.1162/089976602317318965; 10.1002/hipo.20116). (add stuff from ruetishauser where he shows SFC with theta modulates memory?) We therefore hypothesize that neural firing during encoding and retrieval of episodic memories occurs during different phases of the ongoing theta oscillation.

To conclude, we hypothesized that (i) neural firing of ESNs occurs within separate theta phases during encoding and retrieval and that (ii) this episode specific code reflects in a reinstatement of high frequency power in the local field potential while (iii) Concept Neuron related activity is not captured by changes in high frequency power.

We here present evidence for the reinstatement of high frequency power in the local field potential of microwire electrodes between encoding and retrieval of individual episodic memories. Differences between reinstated and non-reinstated episodes suggest that power is broadly increased over higher frequencies and not only within the frequency range of interest (40-200 Hz). As an important control analysis, we do not find high frequency activity increases due to the presence of specific concepts or images, which rules out the possibility that these findings were due to the activity of Concept Neurons.

Results

We studied recordings from two different experiments (experiment 1: 585 neurons and xx microwires in the hippocampus, 16 participants, 7 female; average age = 36.125 years, from 26-53 years; experiment 2: 216 neurons and xx microwires in the hippocampus, 14 participants, 7 female; average age = 33.857 years, from 19-58 years). Patients were implanted with stereotactic Behnke-Fried depth electrodes while completing a memory association task (see xx).

During the encoding phase of experiment 1 patients were instructed to mentally create a vivid story consisting of an animal cue and two associate images (two faces, two places, or a face and a place). There was only one associate image in experiment 2 and cue and associate could be either a face, a place, or an animal. Following a short distractor task where patients had to indicate whether a series of 15 numbers were odd or even the retrieval phase begun. During the retrieval phase the cue image was presented and the patient had to recall the associate image(s). Each episode was learned and retrieved only once and the experiment was self-paced.

In order to investigate high frequency power reinstatement, we calculated the average power within a range of 40 Hz to 200 Hz in steps of 5 Hz for every microwire. During encoding we considered neural activity from the time point the associated image was presented until the patient gave their response. During retrieval the time of interest stretched from the cue onset to the response. We z-scored the power values independently for encoding and retrieval and subsequently excluded episodes that were later forgotten. We defined the element-wise product of the standardized encoding and retrieval power values as a measure of episode-specific reinstatement.

Using a trial-shuffle procedure we re-computed these reinstatement values 1,000 times. If any empirical reinstatement value exceeded the 99th percentile of these permuted values and if the standardized power at encoding and retrieval during that episode exceeded a value of at least 1.645 we considered this microwire an Episode Specific Microwire (ESW).

To estimate how many ESW we can expect by chance we then randomly drew one of the previously calculated permutations for each microwire and applied the same thresholding technique to these shuffled reinstatement values. This allowed us to create a distribution of ESW under the null hypothesis against which we could compare the number of empirically identified ESW. Using this approach, we found a significant number of ESW in experiment 1 (n = 139 out of 1010 microwire, *p* = 0.0310; permutation test). POWERSPECTRA FIGURE. In the second experiment we excluded all episodes that contained an image which reliably evoked a HFB power increase during a visual tuning task. During the visual tuning task, the same images that were previously used in the memory task were shown repeatedly without an episodic memory component. This approach has been traditionally used to detect neurons responding to specific concepts or categories (Florian, Rodrigo xx). We defined Concept Specific Microwires (CSM) as any microwire with a significant increase of HFB in any of 19 overlapping 100ms time bins following the image presentation across all six repetitions in comparison to a 500ms pre-stimulus baseline period using a Mann-Whitney U test (see Methods). When excluding these tuned images, we still found a significant number of ESW in experiment 2 (xxx). In contrast, we did not find a significant number of CSM (xx). To conclude, we found a memory code of HFB power reinstatements between encoding and retrieval of individual episodes in the LFP in two independent experiments. This power reinstatement cannot be explained by a content specific code (i.e., CSM).

We next investigated whether single neuron firing would preferably occur within a specific theta phase during encoding and retrieval of episodic memories and whether there was a neuron specific phase offset between firing during the encoding and retrieval phases.

Based on previous literature no single theta frequency dominates the human hippocampus (xx). Instead, there is a lower theta oscillation (2-5 Hz) and a faster theta oscillation (5-9 Hz).

Unfortunately, we do not know which microwire best represented the dendritic input into a single neuron. Because of that we computed theta components using a weighted average of all microwires within one microwire bundle based on the generalized eigendecomposition of the narrowband theta covariance matrix and the broadband covariance matrix (see Methods).

We distinguished three different categories of activity: spikes of ESN that occurred during reinstated trials (rESN), spikes of ESN during non-reinstated trials (nESN), and spikes of other neurons (SU).

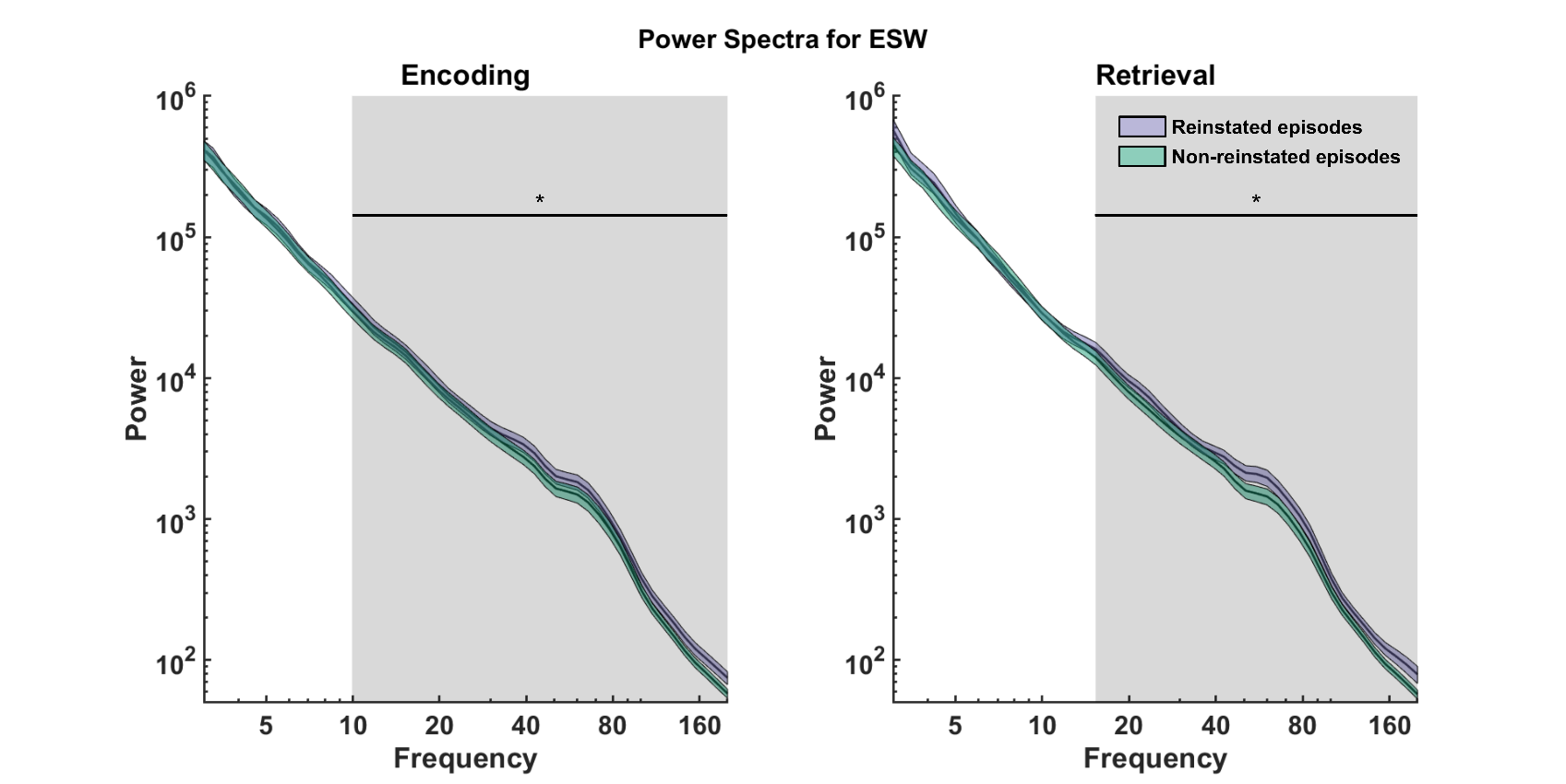
We first computed the preferred mean phase during encoding and retrieval for each neuron. In order to determine a general phase preference, we pooled this prefered phase value over all neurons within a category of neuron (rESN, nESN, SU) and used the Rayleigh test for statistical significant deviations from a uniform phase distribution. In experiment 1 only the SU category showed a phase preference for the slow theta component during encoding (θ = -162.5°, *p* = 0.048) and retrieval (θ = -178.1°, *p* = 0.004). After adjusting for multiple comparisons for two tests (slow and fast theta) SU only show a slow theta phase preference during retrieval (*pencoding adj.* = 0.096; *pretrieval adj.* = 0.008; Bonferroni corrected). Neither rESN nor nESN showed any slow or fast theta phase preference during encoding or retrieval (all *p* > 0.28). In experiment 2 the SU category showed a preference for the slow theta component during encoding (θ = -72.8°, *p* = 0.002; retrieval *p* = 0.633; all other *p* > 0.10). rESN showed a phase preference for the slow theta component during retrieval (θ = -158.7°, *p* = 0.048) that did not survive adjusting for multiple comparisons.

There was a statistically significant phase preference in rESN for the slow theta component during retrieval (θ = -158.7°, *p* = 0.048), however, after controlling for multiple comparisons (slow and fast theta), the effect was no longer significant (*padj.* = 0.096).

It is possible that despite an absence of phase preference during encoding or retrieval, neurons show a reliable offset between the two phases (a representative example of a 10° offset with four neurons: encoding: 0°, 90°, 180°, 270°; retrieval: 10°, 100°, 190°, 280°).

In order to determine if there was a significant theta phase difference between neurons firing at encoding and at retrieval, we computed the mean cosine similarity of the complex value for each neuron for all spikes during encoding with all spikes during retrieval. We determined the statistical significance of the encoding-retrieval phase offset separately for each neuron type (rESN, nESN, SU) using a one-sample test with a mean angle of 0° (i.e., no phase difference between encoding and retrieval). This one-sample test is the circular equivalent of a one-sample t-test with continuous data (we used the function *circ\_mtest* from the Circular Statistics Toolbox v1.21.0.0). In experiment 1 this approach yielded no significant encoding-retrieval phase differences for any category of neurons (rESN, nESN, SU) or theta components (slow, fast) (all *p* > 0.26). Likewise, no encoding-retrieval phase differences were found in experiment 2 (all *p* > 0.4)

To conclude, we find a slow theta phase preference for SU during encoding in experiment 2 and retrieval in experiment 1. However, no neuron type (rESN, nESN, SU) shows a significant encoding-retrieval theta phase offset.



Timeline

Description automatically generated

Materials and Methods

Procedure of memory experiment 1 and experiment 2

See above.

Participants

See above.

Ethical approval

See above.

Behavioural analysis

See Above.

Co-Registering

See Above.

Recording System and Electrodes

See above.

Statistical analysis

All statistical analyses were conducted using MATLAB R2020a on a computer running Windows 10 Enterprise. The significance threshold for all statistical tests was set at 0.05. Unless specified otherwise, all permutation tests were implemented with *N* = 1,000 random draws.

Identification of Episode Specific Neurons (ESNs)

See above.

LFP pre-processing (HFA analyses)

We downsampled the LFP data from microwires that contained neurons in the hippocampus to 1,000 Hz and applied a fourth-order Butterworth bandstop filter with a centre frequency of 50 Hz (± 1 Hz) and its harmonics up to 300 Hz, to remove line noise.

LFP Artefact Rejection (HFA analyses)

For each microwire, we computed the bandpass-filtered signal between 40 Hz and 200 Hz using a first-order Butterworth filter. We identified any data points exceeding five standard deviations from the mean of this signal as artefacts and excluded the one-second intervals preceding and following them.

Identification of Episode Specific Microwires (ESWs)

We considered neural activity from the onset of the associated image to the patient's response in encoding trials, and from the cue onset to the response onset in retrieval trials. To account for edge artefacts, we extended these trial definitions by 100ms on each side. We then performed a wavelet analysis using wavelets from 40 Hz to 200 Hz in steps of 5 Hz and a width of 7 cycles, on the linenoise-removed broadband signal. After removing all artefacts (see #Artefact Rejection), we computed the mean power over all frequencies.

Trials that consisted of 50% or more artefacts during encoding or retrieval were excluded, and if fewer than nine trials remained, the microwire was not considered for further analysis.

We z-scored the remaining HFA power values independently for encoding and retrieval, and afterwards excluded later forgotten trials.

Finally, we defined the element-wise product of the encoding and retrieval standardized HFA power as a proxy for episode-specific reinstatement.

In order to calculate a threshold for this episode-specific firing reinstatement we permuted the order of the encoding and retrieval episodes and recomputed the reinstatement value. We repeated this step 1,000 times and took the 99th percentile as a threshold against which we compared the empirical reinstatement value. If the empirical reinstatement exceeded the threshold and its standardized power at encoding and retrieval was at least 1.645 (≙ pright-tailed < 0.05), we considered this microwire an Episode Specific Microwire (ESW). This procedure allows for thresholding but does not correct for multiple comparisons on the level of a microwire.

To determine whether there was a significant number of microwires that showed an episode-specific power reinstatement, we randomly drew one of the previously calculated permutations for each microwire and determined whether it would be classified as a ESW under the same criteria as before. In each of the 1,000 permutations, we summed up the number of shuffled ESW which we then used to create a null distribution against which we compared the empirically determined number of ESW.

In order to generate Figure XX, we repeated the time-frequency analysis in the range of 3 Hz and 200 Hz for all microwires that exhibited a HFB power reinstatement in at least one episode. For each ESW we calculated the mean power in the HFB during reinstated and non-reinstated episodes and then averaged the respective power spectra across all ESW. To determine the statistical significance of the results, we used a cluster-based permutation test (Oostendorf/feld xx).

Identification of putative Concept Microwires (CWs)

We have adapted the method created by Mormann et al. (2011; 2008 xx) for detecting Concept Neurons to identify microwires whose power in the HFB (xx) was reliably increased following the presentation of a specific image. For each microwire we divided the local field potential of the 1000ms interval post-stimulus into 19 100ms overlapping bins, with the 500ms preceding stimulus onset as the baseline period. To prevent edge artefacts, we extended the testing and baseline intervals by 100ms on either side.

We performed a time-frequency analysis using wavelets in the range of 40 Hz to 200 Hz (stepsize: 5 Hz) and a width of 7 cycles, allowing us to estimate the time-resolved power. We then averaged the power over all frequencies and within each time bin. If more than one of any of the six repetitions of an image contained over 50% artefacts that time bin was discarded for all repetitions.

We then compared the mean HFA power in the remaining 19 bins across all six presentations of an image with the mean HFA power of all baseline periods in the session using a Mann-Whitney U test. We corrected for multiple comparisons using the Simes’ procedure (Rødland, 2006xx). PERMUTATION TEST

Theta components and pre-processing

As a first step, we downsampled the microwire signal to 100 Hz. Because we do not know the relative position of the recorded neurons to the microwires within a bundle of electrodes by extension we do not know if the microwire on which the neuron was recorded best represents the neural input into the neuron. For this reason, we took into consideration all eight microwires and generated two theta components using generalized eigendecomposition (xx).

The generalization of the eigendecomposition extends the eigendecomposition to a case with two square matrices. For an eigenvaluedecomposition with a singular square matrix, the eigenvector with the highest eigenvalue accounts for the maximal variance in the underlying square matrix and is pairwise orthogonal to the other eigenvectors.

In contrast, the eigenvector with the highest eigenvalue in a generalized eigendecomposition can be understood as the filter that maximizes the difference between the two input matrices. The eigenvectors in a GED are independent, but not orthogonal. In practice when applied to two covariance matrices where one matrix represents the broadband activity and the other matrix is generated using a narrowband signal the first eigenvector yields a spatial weighting that maximizes the narrowband activity and minimizes the broadband activity. This eigenvector can be applied to the narrowband filtered multichannel data to get generate a narrowband component. https://doi.org/10.7554/eLife.21792 xx

Based on previous literature (xx) we generated a slower theta component in the frequency range of 2 Hz to 5 Hz and a second, faster component in the range of 5 Hz and 9 Hz. To generate these components, we first applied a first order Butterworth filter to bandpass the broadband signal in all eight microwire channels between 2 Hz and 5 Hz (slow theta component) or 5 Hz and 9 Hz (fast theta component). We then demeaned the signal and computed a covariance matrix using this narrowband signal, which we divided by the number of samples. Next, we computed a second covariance matrix using the entire broadband signal. We computed the generalized eigendecomposition of these two covariance matrices and used the eigenvector with the highest eigenvalue as a spatial filter for the narrowband filtered signal to generate a narrowband component. We then applied the Hilbert transform to the narrowband component get the analytic signal.

We considered the spikes of neurons up to two seconds preceding the patient's response during the encoding and retrieval of later remembered episodes. Each neuron had to contain at least 11 spikes within the time of interest to be included for further analysis. We confined all spike-field analyses to spikes and LFPs that were recorded on the same Behnke-Fried electrode.

We first wanted to estimate phase preference during encoding and retrieval independently. To do this we identified the complex value at the time of each spike. We next normalized each complex value and averaged across spikes. For each neuron with spikes within the time of interest we computed the preferred phase by computing the angle of this average complex number. To estimate phase preference across neurons we performed a Rayleigh test.

For each neuron we determined the complex value of the narrowband component at the time of the relevant spikes during encoding and retrieval. We next investigated whether there was a significant difference in the phase of the narrowband signal between spikes during encoding and retrieval for (i) Episode Specific Neurons in trials that were later reinstated (rESN), (ii) for Episode Specific neurons in trials that were later not reinstated (nESN) and (iii) all other neurons (SU). To this end, we computed the cosine similarity between the complex value of each spike at encoding with the complex value of each spike at retrieval. We then averaged these similarity values across spikes for each eligible neuron. We determined the statistical significance of these difference scores using a one sample test for a mean angle of 0°, which we implemented using the function *circ\_mtest* from the Circular Statistics Toolbox v1.21.0.0).

Discussion

Episodic memories refer to distinctive events that occurred at a specific time and space. These memories are composed of multiple components. In Chapters 1 and 2 we identified the neural basis of how the hippocampus processes these episodic memories. These neurons (called Episode Specific Neurons; ESNs) increase their firing rate during encoding of specific episodic memories and reinstate this firing rate during memory retrieval. In the present chapter we extend these findings from single neurons to the population level by investigating the local field potential (LFP) as a proxy of multi unit activity.

We analysed two independent datasets that were collected using microelectrodes located in the human hippocampus while patients performed a memory association task.

We identified a significant number of microwires that reinstate the power in the high frequency band from encoding to retrieval of specific episodes (HFB; 40-200 Hz). These findings cannot be explained by a content code because (i.e., HFB activity induced by the presence of particular concepts). While the existence of Concept Neurons is undisputed, their activity does not seem to be reflected in the HFB. It is unclear whether the cause of this is the spatial distribution of CN assemblies, their small size or their asynchronous firing.

Our analyses revealed that the power differences between reinstated and non-reinstated episodes exceeded the frequency range of 40-200 Hz that we used to differentiate the two. Reinstated episodes were characterized by an increased power from 10 Hz (during encoding) and 15 Hz (during retrieval), implying that the distinction between reinstated and non-reinstated episodes may not be limited to the HFB, but could be attributed to either an offset or a spectral tilt of the 1/f power spectrum. Future studies will need to carefully disentangle the individual contributions of oscillatory changes, a power offset, and a spectral tilt between reinstated and non-reinstated trials.

There is an ongoing debate as to whether increased HFB power indicates increased MUA firing synchrony or merely more firing. Unfortunately, due to the limited number of single neurons that can be recorded using microwires, we cannot resolve this question using the currently available electrodes. Utilizing hardware such as a Neurapixels probe would allow us to record hundreds of neurons simultaneously, thereby enabling us to examine how HFB power relates to MUA activity during episodic memory processing in the hippocampus.

A considerable body of literature exists that emphasizes the importance of theta oscillations for memory processing (xx). One influential theoretical model proposed that encoding and retrieval of memories occur in opposite phases of the theta oscillation thereby avoiding that encoding new information causes catastrophic interference of older memories (hasselmo, xx).

Recent studies have revealed that there is not one dominant theta frequency in the human hippocampus, but rather two distinct oscillations - a slow (2-5 Hz) and a fast (5-9 Hz) theta oscillation (xx). We investigated how the firing activity of different previously identified neuron types relates to the phase of the ongoing theta oscillations during memory encoding and retrieval. We distinguished between spikes from ESNs during reinstated (rESN), non-reinstated episodes (nESN) and spikes from other single neurons (SU).

Contrary to existing literature we found no consistent evidence that any type of neuronal activity occurred during specific theta phases at encoding or retrieval nor of any reliable difference in theta phase preference between encoding and retrieval.

These unexpected results could be due to various reasons: (1) there is substantial variance between the firing rates of neurons. As a consequence sparse firing neurons might not allow a reliable estimating of phase preference whereas frequently firing neurons fire so often that their

We employed a frequentist approach when analyzing our data; thus, while we did not find compelling evidence to reject the null hypothesis (i.e., no theta phase difference between spikes at encoding and retrieval), this should not be interpreted as evidence for the null hypothesis. To further investigate this, future studies should use a Bayesian framework.

Why no theta findings? While there is some rudimentary evidence that other SU show a encoding and retrieval firing difference

It is interesting that each neuron seems to keep their firing towards a relative theta fast equal between encoding and retrieval

It seems that neurons just fire to their preferred frequency at encoding and retrieval this is especially interesting considering there is mostly no phase preference during encoding and retrieval. Not every neuron might be coding an encoding event so just that there is no phase preference at encoding might not mean that encoding does not occur at any specific phase. Maybe the units we recorded weren’t just involved in the memory encoding process. This is might be true for SU and nESN, but not for rESN. However, there are very few reinstated trials over both experiments which might be not enough data to interpret anything. This problem gets worse if you consider that some neurons are bursty or fire a lot, which muddies the spike field relationship.

These findings extend the discoveries of the previous chapters from the single neuron level to the population activity reflected in the LFP.