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 that is 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). 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).

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). 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.

High frequency activity in the range of 70 Hz to 150 Hz has been used as a proxy for locally confined single neuron activity (xx). Ruetishauser and colleagues (novelty cells xx) reported that 40% of all recorded cells responded to a new stimuls and xx% of all neurons responded to previously shown stimuli in a memory recognition task. It is unclear whether and how many of these neurons reinstate their firing rate and how these findings translate to an episodic memory task. However, based on these findings of task related widespread single neural activity in the hippocampus, we postulate a reinstatement of oscillatory power in the high frequency band from encoding of specific trials to their reinstatement during an episodic memory task. Concept Neurons on the other hand are thought to be part of smaller assemblies of about 160 neurons. We therefore expected not to find changes in high frequency power induced by specific concepts.

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.

HFA notes:

Because the amplitude of single neurons rapidly declines with an increasing distance to the neuron soma with current methods we cannot reliably record single neuron activity further than ~100 μm away (doi.org/10.1529/biophysj.107.111179; doi.org/10.1038/nn1233).

microelectrodes in macaque somatosensory cortex: LFP HFA ~60-200 Hz correlates with average firing rate recorded

40-80hz showed a much smaller correlation. Computational model showed that this increased HFA power can be due to an increased firing rate or neural synchrony (more to the latter) [10.1523/JNEUROSCI.2848-08.2008]

increases in firing rate leads to a broadband increase in the power spectrum (doi.org/10.1371/journal.pcbi.1000609)

Broadband LFP changes correlate with mean firing rate in humans (Manning; doi.org/10.1523/JNEUROSCI.2041-09.2009)

The majority of the recorded LFP in the cortex has been demonstrated to originate from ~250 ym from the recording electrode[xx Katzner S, Nauhaus I, Benucci A, Bonin V, Ringach DL, et al. (2009) Local

origin of field potentials in visual cortex. Neuron 61: 35–41 &

xx Xing D, Yeh CI, Shapley RM (2009) Spatial spread of the local field potential

and its laminar variation in visual cortex. J Neurosci 29: 11540–11549.]

In the visual cortex of the macaque neural firing correlated highly with high gamma power (80-200hz) and the authors suggest broadband high-gamma to be a reliable index of local neural firing. Neural firing likely leads to broadband power shifts but that they can only reliably be detected from >50hz because of 1/f noise. (10.1371/journal.pbio.1000610)

In monkey visual cortex during movie presentation multi unit activity was correlated with gammaband power (30-100hz), but specififcally during the negative going phase of the delta wave (2-4hz) (10.1016/j.neuron.2009.08.016)

10.1126/sciadv.abb0977

Monkey V1 and A1 laminar analysis of broadband high-frequency activity (70-150hz) influence of MUA/local neural firing.

Early-Deep BHA clear spatial and temporalal increase in MUA

Late-superficial BHA more prevalent but only had a weak association with MUA (-> reflects dendritic processes)

While high frequency activity (70-150hz) from deeper layers in the monkey V1 and A1 cortex highly correlated with MUA, the more prevalent superficial HFA

Late-superficial BHA was more prevalent and likely represented dendritic processes rather than MUA activity.

Memory encoding and retrieval led to increased gamma (50-500hz) in humans within the visual pathway and the hippocampus [doi.org/10.1093/brain/awu149]

40–130 Hz

Neural firing rate increases was associated with a higher gamma (40-130hz) lfp power in the auditory cortex (doi.org/10.1016/j.cub.2007.06.066). This relationship was higher when the recorded neurons firing was anticorrelated. However this study contained a small sample of three human subjects (59 neurons).

doi.org/10.1038/nrn3241: buzsaki paper

"Thus, increased power in the higher-frequency bands can be regarded as an index of spiking synchrony."

"high-frequency LFP power can provide a 'proxy' for the assessment of neuronal outputs"

"The 'mesoscopic' information provided by the high-frequency band of the LFP is therefore an important link between the macroscopic-level EEG and the microscopic-level spiking activity of neuronal assemblies."

Most of these findings originate in early sensory processing areas with a sensorotopic structure. In contrast neighboring neurons in the hippocampus represent vastly different things (Rodrigo paper).

Because of this it is conceivable that the relation between HFA and neural firing does not extend to the hippocampus (however, see Manning that and the other hippocampus paper)

Results

Chart, histogram

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 Preprocessing

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

For each microwire, we computed the bandpass-filtered signal between 70 Hz and 150 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 70 Hz to 150 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 trials not of interest (i.e., later forgotten or later remembered 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 Episodic 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 this 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 300 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 70 Hz to 150 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).

%% THETA & POS

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 then 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, (ii) for Episode Specific neurons in trials that were later not reinstated and (iii) all other neurons. 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 Rayleigh test for uniformity.