Title: High frequency power reinstatement in the human hippocampus during episodic memory

Abstract

Previous work has identified single neurons in the human hippocampus that significantly increase their firing rate during the encoding and retrieval of specific episodic memories (Episode Specific Neurons; ESNs). High frequency power (40-200 Hz; HFP) in the local field potential has been used as a proxy for multi unit activity. We here studied the reinstatement of HFP in the hippocampus of patients while they completed a memory association task. Our results suggest a memory code implemented through LFP HFP reinstatement between encoding and retrieval of individual episodes and that this code is not driven by a content-specific code (i.e., population activity of Concept Neurons).

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

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 individual neurons: the local field potential (LFP). In contrast to local neural firing LFPs are a combination of thousands of local and distant transmembrane currents. (xx). We will probe the role of two prominent frequencies in the hippocampus LFP: the theta frequency (2-9 Hz) and activity in the high frequency band (40-200 Hz).

Episodic memories refer to distinctive events that occurred at a specific time and space. These memories are composed of multiple components. In Chapter 1 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 and retrieval of specific episodic memories. 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.

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 ESNs (see Chapter 1) to increase HFP, and further if these neurons are close enough in space and fire in synchrony. Preliminary evidence comes from Rutishauser and colleagues who reported that roughly 20% of all neurons in the hippocampus and amygdala respond to novel stimuli (xx), which is likely 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.

Based on the average number of identified Concept Neurons, recorded neurons, and presented images, it is estimated that approximately one million neurons within the medial temporal lobe code for a given concept. This represents only 0.1% of the total number of neurons in the MTL (10.1038/nrn3251), which likely does not impact HFP.

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 (10.1038/nrn3251) we expect not to find changes in high frequency power induced by specific concepts.

To conclude, we hypothesized that (i) an episode specific code is reflected in a reinstatement of high frequency power in the local field potential while (ii) Concept Neuron related activity is not captured by changes in high frequency power.

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.

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.

To generate Figure XX, we repeated the time-frequency analysis in the range of 3 Hz and 200 Hz in 50 logarithmically spaced steps for all microwires that exhibited a HFP reinstatement in at least one episode. For each ESW we calculated the mean HFP 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 Specific Microwires (CSMs)

We have adapted the method created by Mormann et al. (2011; 2008 xx) for detecting Concept Neurons to identify microwires whose HFP (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).  
To test whether our dataset has a significant number of CSMs for each microwire we shuffled the trial order and recomputed the CSM detection pipeline. We repeated this step 1,000 times to generate a distribution of how many CSM to expect under the null hypothesis.

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.*

Reinstatement of high frequency power

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* = 144 out of 1010 microwire, *p* = 0.0310; permutation test). However, there was no significant number of ESW when limiting the analyses to later forgotten episodes (*p* = 0.305; permutation test). We subsequently contrasted the power spectra of reinstated episodes with non-reinstated episodes from 3 Hz to 200 Hz using 50 log-spaced frequency points. A cluster-based permutation test revealed that during reinstated trials, the power was significantly increased from 9.9 Hz to 200 Hz (*p* < 0.001) at encoding and from 15.3 Hz to 200 Hz (*p* < 0.001) at retrieval (see Figure 1).

HFP reinstatement is not content dependent

The second experiment included a visual tuning task, during which the same images that were used in the preceding memory task were presented repeatedly without an episodic memory component. This approach has been traditionally used to detect neurons responding to specific concepts or categories (Florian, Rodrigo xx) and allowed us to exclude all episodes that contained an image which reliably evoked a HFP increase during a visual tuning task. We defined Concept Specific Microwires (CSM) as any microwire with a significant increase of HFP 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).

We carried out the analysis twice, once with the typically used cut-off threshold of *p* = 0.0005 and again with a more liberal cut-off threshold of *p* = 0.05. Note that no corrections were made for testing multiple images for tunings, thus making a threshold of *p* = 0.05 very liberal.

No CSMs were detected at *p* = 0.0005; however, when the threshold was lowered to *p* = 0.05, we found a significant number of CSMs (*p* = 0.005, permutation test).

Because no CSMs were detected at a cut-off of *p* = 0.0005, no episodes were excluded in the ESW analysis. In experiment 2 we replicated our prior results and found a significant number of ESWs (*n* = 52 out of 339 microwire, *p* = 0.003). We then repeated the ESW analysis, this time excluding episodes with significant CSM activity at a threshold of *p* = 0.05. Despite this threshold change, we identified a significant number of ESWs (*n* = 50 out of 339 microwire, *p* = 0.001).

In summary, we discovered a memory code of HFB power reinstatements between encoding and retrieval of individual episodes in the LFP across two independent experiments. Our findings could not be accounted for by a content-specific code (i.e., CSM). Although we were unable to detect any CSM activity using the traditionally used threshold, we detected a significant number of CSM with a more liberal threshold.

Chart, histogram

Description automatically generated

**Figure XX. Power spectra for reinstated episodes (purple) and non-reinstated episodes (green) during (A) encoding and (B) retrieval.** The x-axis displays the frequency, ranging from 3 Hz to 200 Hz in 50 logarithmically spaced increments. The y-axis displays the power on a logarithmic scale to enhance visibility. The shaded regions show the SEM. The grey rectangles specify frequencies at which the power during reinstated episodes significantly exceed the power of non-reinstated episodes (9.9 Hz to 200 Hz at encoding; 15.3 Hz to 200 Hz at retrieval, based on a cluster permutation test; Maris and Oostenveld, 2007 xx).

Timeline

Description automatically generated

**Figure XX. Five second data snippet showing activity in the slow (2-5 Hz; A) and fast (5-9 Hz; B) components.** Components were generated by taking a weighted average of the narrowband signal of all microwires within a bundle. The weighted average was calculated using a generalized eigendecomposition of the broadband and narrowband covariance matrices.

Chart, histogram

Description automatically generated

**Figure XX. Number of reinstated episodes and number of ESW expected under the null hypothesis.** (A) Pie chart showing the number of episodes each neuron reinstated during experiment 1 (zero episodes: 598 microwire; one episode: 345 ESW; two episodes: 60 ESW; three episodes: 7 ESW; four episodes: 1 ESW). (B) Same as (A), but for experiment 2 (zero episodes: 224 microwire; one episode: 97 ESW; two episodes: 15 ESW; three episodes: 3 ESW). (C) Distribution of the number of ESW expected by chance and the number of empirically found ESW (red line) in experiment 1. (D) Same as (C) but for experiment 2.

Discussion

Episodic memories refer to distinctive events that occurred at a specific time and space. These memories are composed of multiple components. In Chapter 1 we identified the neural basis of how the neurons in the human hippocampus processes these episodic memories. These neurons (called Episode Specific Neurons; ESNs) increase their firing rate during encoding and retrieval of specific episodic memories. 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 (40-200 Hz) from encoding to retrieval of specific episodes. These findings cannot be explained by a content code (i.e., HFP induced by the presence of particular concepts).

Applying the traditional criterion used in Concept Neuron detection to the HFP seems to be too conservative. Despite the relatively small assembly size of Concept Neurons (10.1038/nrn3251), we found a significant number of microwires (CSM) that show a consistent HFP increase to the presentation of specific concepts when lowering this threshold. Importantly, the same threshold was also lowered for the group-level permutation test, which we used to determine the number of CSM expected under the null hypothesis.

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 40-200 Hz, 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 HFP indicates increased MUA firing synchrony or merely more firing (xx). Unfortunately, due to the limited number of single neurons that can be recorded using currently available microwires we cannot resolve this question. Utilizing hardware such as a Neurapixels probe would allow us to record hundreds of neurons simultaneously (xx, xx), thereby enabling us to examine how HFP relates to MUA activity during episodic memory processing in the hippocampus.

To conclude the present chapter, in two independent datasets we identified a significant number of microwires that increase their HFP during encoding and retrieval of specific memories. Although we did not find reliable HFP increases to specific concepts using the traditionally used threshold, we identified a significant number of concept coding microwires using a more liberal threshold. Importantly, the HFP reinstatement for specific memories could not be attributed to this content code. Contrary to our hypothesis, we did not find evidence of neural firing in specific phases during encoding and retrieval, or a phase difference between encoding and retrieval.

NOTES FOR DISCUSSION

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. Correlate each single neuron firing with every frequency in HFP. Future studies should look at individual frequencies between 40 to 200hz. Extend range a bit as well.

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!