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. Consistent with earlier observations we find a significant number of microwires that show a reinstatement of HFP from encoding to retrieval in individual episodes. Importantly, this reinstatement is not driven by a content-specific code (i.e., population activity of Concept Neurons). This effect is limited to later remembered episodes and not present for later forgotten episodes. 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

Episodic memories refer to distinctive events that occurred at a specific time and space. These memories are composed of multiple multimodal elements.

In the previous chapter we investigated the formation and retrieval of these episodic memories on the level of single neurons in the human hippocampus. These neurons (called Episode Specific Neurons; ESNs) increase their firing rate during encoding and retrieval of specific episodic memories. We provided compelling evidence that this episode specific code 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 reflect the aggregate of a myriad of local and distant transmembrane currents (xx). We will focus on the role of high frequency power (HFP; 40-200 Hz) as a proxy of local synchronous spiking activity (xx). Most of the literature examining the relation of spiking activity and HFP 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 HFP-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.

In conclusion, we postulate a reinstatement of 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.

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

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 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 (≙ *p*right-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 (CSWs)

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 CSWs for each microwire we shuffled the trial order and recomputed the CSW detection pipeline. We repeated this step 1,000 times to generate a distribution of how many CSW to expect under the null hypothesis.

Correlation between HFP and spiking activity

After pre-processing the LFP of the microwire on which a neuron was recorded (see #LFP pre-processing) we segmented the data into separate later remembered episodes. During memory encoding the time of interest started at the onset of the associate image(s) and ended when the patient gave their response. In contrast, during memory retrieval, the time of interest started at the cue onset and ended when the patient gave their response. We added 100ms on each side to account for edge artefacts. Then, we performed a wavelet analysis between 40 Hz and 200 Hz in steps of 5 Hz and a width of 7 cycles, and averaged the power across frequencies. We then normalized the HFP across time, using a z-transformation, and concatenated this standardized power values across all episodes. To compute the instantaneous firing rate of the corresponding neuron, we convolved the firing times with a Gaussian kernel (kernel parameters: mu = 0, standard deviation = 50ms, length = 300ms, normalized peak to 1). We then z-scored this instantaneous firing rate and concatenated all episodes. Subsequently, we performed a linear correlation between the concatenated standardized HFP and the concatenated standardized instantaneous firing activity, separately for encoding and retrieval. To assess the statistical significance of the correlation we shuffled the data circularly and recomputed the correlation with this shuffled data. We repeated this step N = 10,000 times and compared the empirical correlation coefficient with the resulting null distribution of shuffled correlation coefficients. We performed this analysis twice: once for neural activity during reinstated episodes and once for all other episodes

Results

We conducted two different experiments in which patients implanted with stereotactic Behnke-Fried depth electrodes completed a memory association task (see xx) In experiment 1 we recorded from 1011 microwires and 585 neurons in the hippocampus (16 participants, 7 female; average age = 36.13 years, from 26-53 years) and in experiment 2 we recorded from 344 microwires and 216 neurons in the hippocampus (14 participants, 7 female; average age = 33.86 years, from 19-58 years). During the encoding phase of experiment 1 patients were instructed to mentally form a vivid story containing an animal cue and two associated images (two faces, two places, or one of each). Experiment 2 only had one associate image and either cue or associate could be a face, a place or an animal. After the encoding phase a short distractor task commenced during which patients had to determine whether a series of 15 numbers were odd or even. During the retrieval phase, the cue image was presented and the patient was asked to retrieve the associated image(s). Each episode was learned and retrieved once and the experiment was completed at the participants' own speed.

Reinstatement of high frequency power

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 microwires, *p* = 0.0310; permutation test; see Figure xx for an example). 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 (CSW) 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 CSWs were detected at *p* = 0.0005; however, when the threshold was lowered to *p* = 0.05, we found a significant number of CSWs (86 out of 344 microwires, *p* = 0.005, permutation test). Because no CSWs 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 344 microwires, *p* = 0.003). We then repeated the ESW analysis, this time excluding episodes with significant CSW activity at a threshold of *p* = 0.05. Despite this threshold change, we identified a significant number of ESWs (*n* = 50 out of 344 microwires, *p* = 0.001). Of note, although the more liberal CSW threshold led to the identification of fewer ESWs the resultant p-value is lower. This is because the reduced threshold is also applied when determining the number of permuted ESWs (i.e., ESWs expected by under the null hypothesis).

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

HFP correlates with ESN and single neuron firing

Next, we examined the correlation between HFP and single neuron firing in our sample. We first determined the instantaneous firing rate of each ESN during reinstated episodes. In a separate analysis we calculated the instantaneous firing rate during non-reinstated episodes. We segmented the LFP data into later remembered episodes and performed a wavelet analysis from 40-200 Hz. For each episode we averaged the power in that frequency range. We then z-scored the instantaneous firing rate and the HFP estimate across time. Finally, we concatenated each episode separately for encoding and retrieval. We performed a linear correlation between the standardized HFP and the standardized instantaneous firing rate and assessed the statistical significance by comparing it with the correlation values that we obtained through circular shuffling. In experiment 1, HFP and ESN firing during reinstated episodes correlated with r = 0.xx during encoding (R² = 0.xx, p = 0.xx; permutation test) and r = 0.xx during retrieval (R² = 0.xx, p = 0.xx; permutation test). Firing during non-reinstated episodes significantly correlated with HFP during encoding (r = 0.xx, R² = 0.xx, p = 0.xx; permutation test) and during retrieval (r = 0.xx, R² = 0.xx, p = 0.xx; permutation test). A similar relationship was found in experiment 2, where HFP and firing during reinstated episodes correlated with r = 0.140 at encoding (R² = 0.xx, p = 0.xx; permutation test) and r = 0.135 during retrieval (R² = 0.xx, p = 0.xx; permutation test). Firing during non-reinstated episodes correlated with HFP with r = 0.xx during encoding (R² = 0.xx, p = 0.xx; permutation test) and r = 0.xx during retrieval (R² = 0.xx, p = 0.xx; permutation test).

**Chart

Description automatically generatedFigure XX. Example ESW.**

(A) The bar plots show the z-scored HFP on the y-axis for 23 episodes on the x-axis colour coded for encoding (blue) and retrieval (orange). The transparent bar encompassing the standardized HFP represent their element wise product, which is used as a measurement for episodic memory reinstatement. The dotted line represents the threshold which is calculated based on a permutation test.

(B) The time resolved HFP (y-axis) during memory encoding with the time in seconds (x-axis) starting from the associate image onset for reinstated episodes (purple) and non-reinstated episodes (green). The shaded area represents the SEM.

(C) Same as (B), but during retrieval and starting at the cue onset.

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 microwires; one episode: 345 ESWs; two episodes: 60 ESWs; three episodes: 7 ESWs; four episodes: 1 ESWs).

(B) Same as (A), but for experiment 2 (zero episodes: 224 microwires; one episode: 97 ESWs; two episodes: 15 ESWs; three episodes: 3 ESWs).

(C) Distribution of the number of ESWs expected by chance and the number of empirically found ESW (red line) in experiment 1. (D) Same as (C) but for experiment 2.

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).

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 how 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 extended 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. Power in the high frequency band (40-200 Hz) on a significant number of microwires was reinstated 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 to in Concept Neuron detection to detect a content code seems to be too conservative to detect significant increases in the HFP. However, when lowering this threshold, we found a significant number of microwires that show a consistent HFP increase when presenting specific concepts (CSW) despite the relatively small assembly size of Concept Neurons (10.1038/nrn3251). Importantly, the same threshold was also lowered for the group-level permutation test, which we used to determine the number of CSW expected under the null hypothesis. Concept Neuron activity might be reflected in the HFP due to the spatial clustering of Concept Neurons within the vicinity of a microwire (but see xx; neighbouring neurons often code something different). Alternatively, multiple Concept Neurons coding the same concept might be active within a short delay, leading to a higher deflection in the LFP. The development of new electrodes that enable the recording of multiple neurons that code the same concept at more precisely known locations (such as xx) might offer an answer to this question.

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.

The range of high frequency activity (40-200 Hz) overlaps with the so-called ripple band (80-120 Hz). Excitatory input from CA3 induces ripple activity in the CA1, which is characterized by highly synchronized neural firing (Buzsaki, 2015). Ripple activity has been linked to memory consolidation and replay of previous experiences (Vaz, 2019, science; Jadhay et al., 2012 science; Roux et al., 2017 nat neuro). For example, research by Vaz and colleagues (Vaz et al., 2019, Science), has implicated coupled ripple activity to coordinate the information flow between the MTL and the temporal cortex (also see Ngo et al., 2020 elife). Considering this, future studies should investigate the extent to which the here reported HFP memory reinstatement effect is driven by activity in the ripple range. Here we report a significant correlation between spiking of individual neurons and the mean power in the high frequency range from 40 Hz to 200 Hz. However, this correlation is weak, explaining only ~1-2% of variance. **Of note, we correlated the firing rate of only a single neuron with the HFP of the microwire on which it was recorded (lots of redundancy with 2 sentences prior).** Since the firing rate reflects just a single neuron, the HFP includes activity from disproportionally more neurons with different firing rates. We therefore expect a higher correlation when analysing more neurons. Moreover, it is conceivable that different microwires from the same bundle may be better suited to pick up transmembrane currents from nearby neurons. Additionally, there is substantial variance in the literature which frequency range constitutes the high frequency band (e.g., xxx). Future studies should aim to identify which frequencies are most indicative of single neuron firing taking into considerations differences between neuron types (excitatory or inhibitory cells). Furthermore, recent research has suggested that synchronous firing is more influential in increasing HFP compared to firing alone (xx). Unfortunately, due to the limited number of single neurons that can be recorded using currently available microwires we cannot resolve this question. Recording more neurons using newer electrodes may enable us to disentangle the roles of synchrony and firing in relation to HFP in the future. A larger brain coverage would also allow the investigation of what role the recorded brain area plays in moderating the relationship between neural firing and HFP (paper where different brain layers produce different findings xx).

To conclude the present chapter, consistent across two independent datasets we identified a significant number of microwires which show a HFP reinstatement during encoding and retrieval of specific memories (ESW). This HFP activity showed a low, but significant correlation with neural firing of ESNs and single neurons during encoding and retrieval. 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 (CSW). Importantly, the HFP reinstatement for specific memories could not be attributed to this content code. Taken together the present work extends findings from the level of the single neuron and provides a potential link to surface EEG recordings (doi.org/10.1038/nrn3241).