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Theta-alpha connectivity in the hippocampal-entorhinal circuit predicts working memory load

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Abstract

Working memory (WM) maintenance relies on multiple brain regions and inter-regional communications. The hippocampus and entorhinal cortex (EC) are thought to support this operation. Besides, EC is the main gateway for information between the hippocampus and neocortex. However, the circuit-level mechanism of this interaction during WM maintenance remains unclear in humans. To address these questions, we recorded the intracranial electroencephalography (iEEG) from the hippocampus and EC while patients (N=13, 6 females) performed WM tasks. We found that WM maintenance was accompanied by enhanced theta/alpha band (2-12 Hz) phase synchronization between the hippocampus to the EC. Granger causality and phase slope index analyses consistently showed that WM maintenance was associated with theta/alpha band-coordinated unidirectional influence from the hippocampus to the EC. Besides, this unidirectional inter-regional communication increased with WM load and predicted WM load during memory maintenance. These findings demonstrate that WM maintenance in humans engages the hippocampal-entorhinal circuit, with the hippocampus influencing the EC in a load-dependent manner.

Significance Statement

Hippocampus is known to be part of the working memory (WM) network. How does the hippocampus communicate with other brain regions to maintain WM information? Rodent studies suggest that hippocampal-entorhinal communication supports WM maintenance. However, it remains unclear whether and how the human hippocampus and EC coordinated during WM tasks. In this study, by combining machine learning analyses with intracranial electroencephalography (iEEG) recordings, we found that WM maintenance is associated with theta/alpha band (2-12 Hz) unidirectional influence from the hippocampus to the EC. The unidirectional inter-regional communication during WM maintenance increased with WM load and predicted WM load. These findings indicate the hippocampal-entorhinal directional coupling as a further element of the WM network.

Introduction

Cognition critically depends on the ability to maintain information in an active state for a short time, which is typically ascribed to working memory (WM)(Baddeley, 2007). Studies found persistent single-neuron spiking (Kaminski et al., 2017; Kornblith et al., 2017; Boran et al., 2019; Boran et al., 2022) and elevated oscillatory activity (Li et al., 2022) in the hippocampus during WM maintenance. An increasing number of studies have pointed out that WM maintenance relies on multiple brain regions (Christophel et al., 2017) and is supported by inter-regional communication (Yamashita et al., 2018; Mamashli et al., 2021; Dimakopoulos et al., 2022). Given the role of hippocampus in WM and the distributed nature of WM, understanding the connectivity between the hippocampus and the rest of the brain could provide a crucial insight into the network involved in such a fundamental process. Then, one may ask how does the hippocampus interact with another/other brain area(s) during WM maintenance, and which brain area(s) contribute to this process?

The Entorhinal cortex (EC) is a key candidate to interact with hippocampus for the following reasons. First, persistent firing during WM maintenance has been consistently observed in EC neurons across rats (Young et al., 1997), monkeys (Suzuki et al., 1997) and humans (Boran et al., 2022). Second, the EC serves as an interface between the hippocampus and cortical/subcortical areas (Lavenex and Amaral, 2000). Third, structural and functional hippocampal-EC interactions have

been extensively reported, involving anatomical connections (Rosene and Van Hoesen, 1977; Small et al., 2011), sensory information transfer, and memory-associated activity feedback (Buzsáki and Tingley, 2018; Rozov et al., 2020). Rodent studies suggest that hippocampal-EC communication supports WM maintenance, as evidenced by synchronized oscillations during WM execution (Yamamoto et al., 2014) and WM impairments upon inhibition of this circuit (Suh et al., 2011; Yamamoto et al., 2014). However, these animal studies have not been validated in humans, partly due to limitations in noninvasive recording methods' spatial and temporal resolution. The hippocampal-EC circuit is crucial in spatial navigation (Zhang et al., 2013), and recent research has extended its involvement to memory-guided behaviors (Squire, 1992). Building on these findings, our study investigates the role of this circuit in WM, a fundamental cognitive process with broad implications (Baddeley, 2012).

If the hippocampal-EC circuit contributes to WM, understanding the neural mechanisms underlying this process is crucial. Theta/alpha oscillations (2-12 Hz), commonly observed in the human medial temporal lobe (Fell et al., 2011; Colgin, 2016), have been implicated in WM. Synchronized oscillations are proposed as a fundamental mechanism supporting inter-regional neural communication (Fell and Axmacher, 2011), and low-frequency phase synchronization between the hippocampus and cortex has been reported during WM maintenance, increasing with

WM load (Boran et al., 2019; Dimakopoulos et al., 2022). Granger Causality (GC) and phase slope index (PSI) are popular techniques used to estimate the directionality of inter-regional interactions. Previous research has reported the role of theta/alpha-gamma phase-amplitude coupling (PAC) in WM maintenance (Roux and Uhlhaas, 2014). However, most studies have focused on PAC within a single brain region, like the hippocampus (Axmacher et al., 2010), with limited inter-regional exploration (Wang et al., 2021). Theta-gamma interactions have been observed in hippocampal-EC communication in rodent studies (Buzsáki, 2002; Hasselmo et al., 2002). The involvement of inter-regional low-frequency synchronization and cross-frequency coupling in the hippocampal-EC circuit during WM processing remains unclear.

Leveraging the high spatiotemporal resolution of iEEG recordings and the analytical power of multivariate machine-learning analysis, we tested the hypothesis that low frequency and gamma oscillations cooperatively facilitate hippocampal-EC interactions to support the maintenance of WM information in humans. We simultaneously recorded iEEG data from the hippocampus and EC in 13 epilepsy patients while they performed a modified Sternberg task (Michels et al., 2008; Boran et al., 2019; Li et al., 2022). Our goal was to address the following questions: (a) Do the hippocampus and the EC interact while humans perform a WM task? (b) Which specific oscillatory modes of interregional communication, including frequency and

directionality, mediate WM maintenance? And (c) Do these interaction modes have functional effects?

Materials and methods

Participants

We used data from 13 adult human patients (mean ± SD [range]: 35±13 [18-56]; 6 females) in this study. All patients were implanted with depth electrodes (1.3 mm diameter, 8 contacts of 1.6 mm length, and 5 mm spacing between contact centers; Ad-Tech, Racine, WI, www.adtechmedical.com) in the medial temporal lobe for evaluation of the surgical treatment of epilepsy. Electrode placement was exclusively guided by clinical needs. There were no seizures recorded during any of the recording sessions, and any trials with interictal epileptiform activity were excluded from analysis. All patients provided written informed consent before participating. This study has been approved by the relevant institutional ethics review board (Kantonale Ethikkommission Zürich, PB 2016-02055), and is in agreement with the Declaration of Helsinki.

Experimental design

We used a modified Sternberg task in which the encoding of memory contents, their maintenance, and their recall were temporally separated (**Fig. 1A**). Each trial started

with a fixation period (1 s) followed by the stimulus for 2 s. Participants were instructed to memorize a set of four, six, or eight letters that were presented at the center of the screen. The number of letters indicated the memory load. After the disappearance of the stimulus, the maintenance interval followed (3 s). After presentation of the probe letter, the participants responded with a button press ("IN" or "OUT") to indicate whether the probe was part of the stimulus letter set. After the response, the participants were encouraged to relax before they initiated the next trial. The participants performed 50 trials per session, which lasted approximately 10 min. During the recording period of several days, several participants performed more than one session of the task up to a total of eight sessions. **Table 1** contains detailed information about the number of trials and sessions for each participant. To eliminate any potential confusion arising from mixing categories with varying proportions of incorrect trials, subsequent analyses exclusively utilized trials with correct responses.

Channel localization and selection

Channel localization was performed using postimplantation computed tomography (CT) scans and structural T1-weighted MRI scans. For each patient, the CT scan was co-registered to the postimplantation scan, as implemented in the FieldTrip toolbox (Oostenveld et al., 2011). The channels were visually marked on the coregistered CT-MR images. The channel positions were then normalized to the MNI 152 space and assigned to specific brain regions using the Brainnetome Atlas (Fan et al., 2016). Channel positions were verified by the neurosurgeon (L.S.) after merging pre-

operative MRI with postimplantation CT images of each individual patient in the plane along the electrode (iPlan Stereotaxy 3.0, Brainlab, München, Germany).

For each participant we analyzed the iEEG from a maximum of three electrodes per hemisphere targeting the hippocampal head (anterior), the hippocampal body (posterior), and the entorhinal cortex. Targeted regions and hemispheres varied across participants for clinical reasons and included the hippocampus in the left (n = 12) and right (n = 13) hemisphere and the entorhinal cortex in the left (n = 12) and right (n = 11) hemisphere. We selected the two most medial channels on each electrode targeting the hippocampus or the entorhinal cortex as in previous studies (Oehrn et al., 2014; Pacheco Estefan et al., 2019). The final number of selected channels in each region for each participant is listed in **Table 1**. We included only ipsilateral channel pairs in the analysis. The final dataset contained 87 channels in the hippocampus and 46 channels in the entorhinal cortex across all patients. There were 6.7±1.5 (range 4-8) channels per patient in the hippocampus and 3.5 ± 0.9 (range 2-4) channels per participant in the entorhinal cortex. **Fig.1C** presents all recording locations with the BrainNet Viewer toolbox (Xia et al., 2013) in MATLAB (MathWorks, Natick, MA).

Data acquisition and preprocessing

Intracranial data were acquired using a Neuralynx ATLAS recording system, sampled at 4 kHz, and analog-filtered above 0.5 Hz. The data were recorded against a common reference where the outermost electrode contact in temporal cortex served as

electrical reference. After data acquisition, neural recordings were downsampled to 1 kHz and band-pass filtered from 1 to 200 Hz using the zero-phase delay finite impulse response (FIR) filter with a Hamming window. Line noise harmonics were removed using a discrete Fourier transform. The filtered data were manually inspected to mark any contacts or epochs containing epileptiform activity or artifacts for exclusion and were then re-referenced. The continuous data were segmented into 8 s trials with a 1 s fixation period as the baseline, 2 s encoding period, 3 s maintenance period, and 2 s retrieval period. We here focused on the maintenance period. The trials with residual artifacts were rejected after visual inspection. In total, we rejected 65 trials with load 4 (5.5%), 36 trials with load 6 (3.9%) and 39 trials with load 8 (5.1%) across all participants. Preprocessing routines were performed using the FieldTrip toolbox (Oostenveld et al., 2011) and customized scripts in MATLAB.

Statistical analyses

To assess the significance of a value, we created a null distribution estimated from 1000 permutations on data with scrambled labels using a non-parametric permutation test. The significance was defined as exceeding the threshold that obtained from the 95^{th} percentile of the empirically estimated null distribution.

To compare the metrics of phase synchronization, GC, PAC and PSI between two loads (load 4 vs load 6, load 4 vs load8, load 6 vs load 8), the statistical significance was then estimated using a permutation test, in which a null distribution was created by randomly assigning trials into two loads, computing the differences between loads,

223 and repeating this procedure 1000 times. We also applied paired *t*-tests to directly
224 compared measurements from the two directions within each load condition. Multiple
225 comparisons were corrected by False Discovery Rate (FDR). $P < 0.05$ was considered
226 significant.

227

228 To rule out potential confounding effects of aperiodic activity on WM load, we first
229 performed separate repeated-measures analyses of variance (ANOVAs) for the
230 hippocampus and the EC, with a within-group effect of load across the frequency
231 spectrum. The aperiodic activity was chosen as it captures non-oscillatory effects at
232 specific frequency, allowing us to examine the precise frequency ranges sensitive to
233 load effect on non-oscillatory components. Additionally, we applied a linear-mixed
234 effect model to explore whether the aperiodic activity from the hippocampus and the
235 EC contributed to the hippocampus-EC interaction, with load, the aperiodic activity as
236 fixed factors, and participants as a random factor, and the electrophysiological
237 indexes as dependent variables.

238

239 For all the decoding analyses, we used a non-parametric permutation approach to test
240 the significance of the accuracy values. We created a null distribution of the decoding
241 accuracy by shuffling the data labels 1000 times. For each decoding analysis, the null
242 distribution was generated for each test and we took the maximum value of the null
243 distributions across tests as a final null distribution for multiple corrections, as
244 previous study did (Mamashli et al., 2021). The averaged decoding accuracy
245 exceeding the 95^{th} percentile of such null distribution ($p < 0.05$) was considered
246 significant.

Time-frequency analysis

Time-frequency power was separately computed within the hippocampus and the EC for trials with different WM loads. For each trial and each channel, we convolved the signal with complex-valued Morlet wavelets (6 cycles) to obtain power information at each frequency from 1 to 100 Hz (in steps of 1 Hz) with a time resolution of 1 ms. The task-induced power was analyzed per trial using a statistical bootstrapping procedure (methods have been described in more detail in our previous publication (Li et al., 2022)). Then, the raw power for each time point during the task was *z*-scored by comparing it to the null distribution to generate the *z*-scored power. For each participant, the *z*-scored spectral power in the theta/alpha band was averaged across the maintenance period within the hippocampus and the EC separately for each WM load.

Previous study reported that periodic properties of electrophysiological data are highly variable, and also coexist with variable and dynamic aperiodic activity (Donoghue et al., 2020a; Donoghue et al., 2020b; Donoghue and Watrous, 2023). To exclude possible confounding effect of aperiodic activity on neural oscillations of the hippocampus and EC, we have adopted a distinct approach by separately characterizing the aperiodic properties of power spectra originating from both the hippocampus and the EC, with the Fitting-Oscillations-and-One-Over-F (FOOOF) toolbox (Donoghue et al., 2020b). We isolated the aperiodic component of power spectra across the entire frequency spectrum for each load and region, and compared

these components among WM load for each region with the repeated-measures ANOVA.

Phase-locking value

To explore the potential interaction between the hippocampus and the EC during WM maintenance, we employed phase-locking value (PLV) to assess the degree of consistency for each channel pair phase relationship independent of their absolute phases and amplitudes among repeated trials, with PLV = 1 referring to strong phase synchrony where all trials are synchronized without any variations between two channels. Using the same parameters of time-frequency analysis, we computed the PLV in the time-frequency domain from 1-100 Hz during maintenance for each channel pair within the same hemisphere (one channel from the hippocampus and one from the EC) for the trials with each WM load.

To evaluate the statistical significance of PLV, a null distribution was created by randomly shuffling the trials with load8 for each channel pair and computing the corresponding PLV spectrogram and repeating the same procedure for 1000 times, as our previous study did (Boran et al., 2019). Then the null distribution of all channel pairs was averaged and only the time-frequency PLV above the threshold (95% of the null distribution) were kept as significant PLV for further analyses. In addition, the workload-dependent increases in the PLV were subsequently assessed by subtracting

the PLV for trials with one load from the PLV for another load, and the statistical significance between two loads was then estimated using a permutation test, as mentioned in the section of statistical analyses.

Granger Causality analysis

After establishing the phase synchrony, which measures undirected connectivity between the hippocampus and EC, we proceeded to investigate the directionality of their interaction using two complementary measures: a frequency-domain GC and PSI. GC measures the degree to which the signal from a region (i.e., the hippocampus) can be better predicted by incorporating information from another signal (i.e., the EC) in a specific frequency band, and vice versa (Zheng et al., 2019). For each channel pair, the trial-wise mean was subtracted from each trial before fitting to an autoregressive model and computing the spectral GC. We then used the Multivariate Granger Causality Matlab Toolbox (Barnett and Seth, 2014) based on the Akaike information criterion to define the model order for each pair. Based on the observations from the PLV above, we computed the GC index across 2-12 Hz (in steps of 0.25 Hz) for both directions (from the hippocampus to the EC and the reverse direction) with the trials of load 4, load 6 and load 8, separately. To test the statistical significance of GC, we created a null distribution by randomly shuffling the signal between the channel pairs 1000 times and averaged the null distribution of all channel pairs. Only the value above the threshold (95% of the null distribution) was kept as significant GC for further analyses. For the GC index across 2-12 Hz from the

hippocampus to the EC as well as the opposite direction, we also applied the permutation test for comparisons between two loads, as mentioned in statistical analyses. To rule out the bias of aperiodic activity, with the linear mixed-effect model, we considered WM load, the aperiodic activity of the hippocampus and the EC within 2-12 Hz as fixed factors, and the participants as a random factor. The hippocampal driven GC as well as the EC driven GC across 2-12Hz was set as the dependent variable.

Phase slope index analysis

On the other hand, PSI examines whether the slope of the phase differences between the channel pairs remains consistent across several adjacent frequency bins (Nolte et al., 2008). A positive PSI signifies that the channel in the first structure (e.g., hippocampus) leads the channel in the second structure (e.g., EC), whereas a negative PSI indicates the reverse. For the trials with WM load, using the FieldTrip toolbox (Oostenveld et al., 2011), the data segments during maintenance were zero padded and multiplied with a Hann taper from 2 to 12 Hz with 1 Hz step, from which we computed the theta/alpha PSI at each channel pair within the same hemisphere in each participant (i.e., one from the hippocampus and the other from the EC) and pooled all possible channel pairs between the hippocampus and EC for each participant. To correct for any spurious results, we randomly shuffled the trials and recomputed the PSI at each channel pair. This step was repeated 1000 times to create normal distributions of channel pair-resolved null PSI data.

To construct a directional effect of the hippocampus-EC on a population level, we averaged the raw PSI across channel pairs and participants. Correspondingly, the null distributions were also averaged across channel pairs and participants. Consequently, the raw PSI outputs can be compared to the distribution of null PSI to derive a *z*-score in the theta/alpha band (for a similar approach, see (Solomon et al., 2019)). To examine if the null distribution of PSI by randomization is a normal distribution, we assessed the normality of the null distribution for different WM loads using the Jarque-Bera test, a widely used statistical test that examines the skewness and kurtosis of a sample to determine its normality. The null distribution of PSI for load4, load6, and load8 is normally distributed (Jarque-Bera test: load4, $p = 0.13$; load6, $p = 0.11$; load8, $p = 0.50$). As a result, raw PSI outputs were *z*-scored and significant PSI was thresholded at $|z| > 1.96$, in which the hippocampus leads were defined as $z > 1.96$ and the EC leads as $z < -1.96$ as in our previous study (Li et al., 2022; Li et al., 2023b).

To assess the statistical significance of PSI differentiation for WM load, the permutation test described previously was also used here to create a null distribution of PSI differences between two loads. Then the real PSI differences were obtained between loads and were then compared with the corresponding null distribution to estimate a *z*-score with the positive value indicating PSI increase in the high load condition versus the low load condition.

Cross-regional phase-amplitude coupling

Next, we investigated the cross-regional PAC between the hippocampus and the EC using the modulation index (MI) (Tort et al., 2010; Vaz et al., 2017), which reflects

the coordinated activity between brain regions. We first calculated the time series of phase and amplitude envelope. This was achieved by applying the standard Hilbert transform to the low-frequency (2-30 Hz) phase, extracted from the hippocampus/EC channel, using a 2 Hz step. Similarly, the high-frequency (30-150 Hz) amplitude was obtained from the channel in the other structure (EC/hippocampus) with a 5 Hz step. Subsequently, we partitioned the continuous phase values of the modulating frequency into 20 evenly spaced phase bins. For each phase of the low-frequency modulating signal, we determined the corresponding amplitude of the high-frequency modulated signal and assigned it to the respective phase bin. To assess this coupling, we employed the MI that quantifies the disparity in entropy between the computed phase-amplitude distribution and a uniform distribution using a normalized Kullback-Leibler distance between each pair of low modulating frequencies and high modulated frequencies.

To assess the statistical significance of PAC, we generated a null distribution with a trial shuffling procedure. Specifically, we created shuffled versions by associating the phase series of trial *k* with the amplitude series of trial *l*, with *k* and *l* randomly chosen among the trial numbers. We then generated 1000 surrogate MI values, from which we could infer the MI chance distribution. To construct a directional effect of PAC on a population level, we averaged the raw PAC across channel pairs and participants. Correspondingly, the null distributions were also averaged across channel pairs and

participants. Consequently, the raw PAC outputs can be compared to the distribution of null PAC to derive a *z*-score in the phase and amplitude frequency band. Significant zPAC was thresholded at $|z| > 1.96$.

We also examined the relationship between cross-regional theta/alpha-gamma zPAC and WM load with the permutation tests for comparisons between two loads. Thresholding was performed at the 95^{th} percentile level, as stated in the statistical analyses section. Similarly, as done in Granger causality analysis, the linear mixed-effect model was applied to examine the effect of the aperiodic activity on zPAC. Specifically, we treated WM load, the aperiodic activity of the hippocampus (2-12 Hz) and of the EC (30-100 Hz) as fixed factors, and the participants as a random factor. The hippocampal theta/alpha phase - EC gamma amplitude zPAC as well as the opposite direction were set as the dependent variables. We also tested the impact of phase synchrony to the functional effect of zPAC within the same model, as previous study conducted (Wang et al., 2021).

Machine learning analyses

In addition to conventional univariate analysis, multivariate analysis detects subtle load-related distribution pattern changes missed by univariate methods (Grootswagers et al., 2017), and enhances findings' generalizability and reliability through inter-subject validation (Wang et al., 2020). Therefore, we next investigated whether the

neural activity and inter-regional communication within the hippocampal-EC circuit was modulated by WM load. We used the patterns from PLV, GC, zPAC, and *z*-scored power from the trials with WM load as our features. Here we used support vector machine (SVM) (Chang and Lin, 2011) as a classifier to classify the WM load (load 4/6/8). SVM is widely used in decoding analyses in neuroimaging studies (Mamashli et al., 2021) because of its suitability for analyses with a relatively small number of samples. It is provided by the COSMOMVPA package (Oosterhof et al., 2016) in MATLAB. And the approach of leave-one-out cross-validation (LOOCV) was applied to validate the decoding accuracy. Considering the inherent difficulty of generalizing across different subjects (Poldrack et al., 2009; Poldrack, 2011), leave-one-out cross-validation (LOOCV) is shown to be a suitable method for obtaining dependable accuracy estimates, especially when working with datasets that have a restricted number of samples (Wong, 2015). Details of our multivariate pattern analysis (MVPA) decoding analyses were as follows:

(A) PLV patterns: We considered the theta/alpha (2-12 Hz) PLV patterns between the hippocampus and the EC during maintenance to investigate whether the phase synchrony pattern could decode the WM load. For each participant and each load, there were *M* features (11 frequency bins × 3000 time bins) converted to a feature vector. We trained a linear SVM classifier and applied LOOCV at subject level by splitting the data set of all subjects (N = 14) into a training set from N-1 subjects and a testing set from the remaining one subject. This process was repeated N times to

ensure comprehensive validation. For each iteration, we used the feature vectors labeled as load 4, load 6 and load 8 from the training dataset, which encompassed the data of N-1 participants. This resulted in a training dataset including (N-1) participants × 3 sets × M features. Subsequently, we calculated the average classification accuracy by averaging the results across the N repetitions of the cross-validation procedure. Meanwhile, to reduce the feature dimensionality, principal component analysis (PCA) was applied to the training data set to keep several principal components (*K* components) that explained 99% of the variance in the data. We also applied the *K* components matrix on the remaining data set from one participant and tested the SVM classifier. This procedure was replicated *N* times for cross-validation. The schematic of the MVPA using the feature patterns is shown in **Fig. 1D**. The accuracy of the classifier was averaged across *N* cross-validations as a measure of performance. To test the significance of the accuracy, we created a null distribution by shuffling the training labels 1000 times. And the averaged decoding accuracy exceeding the 95^{th} percentile of the null distribution ($p < 0.05$) was considered significant.

(B) GC patterns: We considered the GC patterns from two directions, from the hippocampus to the EC and the reverse direction, to allow us to investigate whether there was a specific information flow pattern that could decode the WM load. The GC patterns were calculated in the theta/alpha band separately for trials with different WM load. For each participant and each load, the GC pattern included *M* values (*M* = 41) and these values were converted into a feature vector. As described above, we

used the feature vectors labeled as load 4, load 6 and load 8 from *N-1* participants as a training data set (*N-1* × *3* sets × *M* features) and tested these on the remaining one participant data set. Similar to the LOOCV performed in the previous analysis, we left one participant out for validation and replicated this procedure *N* times. The accuracy of the classifier was averaged across all replications. In total, we separately performed this classification process for the 2 directions: for the hippocampus modulating the EC and for the EC modulating the hippocampus. Similar to the PLV patterns, we generated a null distribution with each direction of GC patterns and took the maximum value of the two null distributions as the final null distribution for multiple corrections, as previous study did (Mamashli et al., 2021). The averaged decoding accuracy exceeding the 95th percentile of the null distribution ($p < 0.05$) was considered significant.

(C) *Z*-scored PAC patterns: We calculated the theta/alpha phase-gamma band zPAC between the hippocampus phase modulating the EC amplitude and the opposite direction differences for the trials of load 4, load 6 and load 8 separately. For each participant and each load, there were *M* features (11 phase bins × 36 amplitude bins) converted to a feature vector. Similar as described for the PLV patterns, we combined *N-1* participants' data set from trials with WM load as training data set, applied zPCA to the training data set to K components that explained 99% of the variance, fed the features (*N-1* × 3 sets × *K* components) into a linear SVM classifier and trained the classifier, and tested it on the remaining one participant data set that already applied *K* components matrix to the testing data set. The accuracy of the classifier was averaged across *N* replications. To test the significance of the accuracy, we created a null distribution by shuffling the training labels 1000 times. And the averaged decoding

accuracy exceeding the 95th percentile of the null distribution ($p < 0.05$) was considered significant.

(D) *Z*-scored power patterns: To address whether local activity in the hippocampus and the EC contributed to WM load, we used a frequency specific *z*-scored power pattern at the theta/alpha band (2-12 Hz, 11 frequency bins) during maintenance from the hippocampus and EC to decode the WM load. The training data set for the linear SVM classifier from *N*-1 participants (*N*-1 × *3* sets × *M* features) and the classifier was tested on the remaining one participant data set. The accuracy of the classifier was averaged across *N* replications by LOOCV. We performed 2 classifications (2 regions) in this decoding analysis and the statistical analysis was performed aligns with the above analyses.

Results

Task, behavior and recording channels

Thirteen patients with drug resistant epilepsy (6 female) performed a modified Sternberg WM task during an invasive presurgical evaluation. In this task, the items were presented simultaneously rather than sequentially, thus separating the encoding period from the maintenance period. In each trial, the participant was asked to memorize a set of four, six, eight letters presented for 2 s (encoding). The number of letters was thus specific for the memory load (load4, load6 and load8). After a delay (maintenance) period of 3 s, a probe letter was presented and the participant

484 responded whether the probe letter was identical to one of the letters held in memory
485 (retrieval) (**Fig. 1A**). Across all sessions, participants' averaged capacity was 7.2,
486 calculated by Cowan's formula (Cowan, 2001), which indicated that the participants
487 were able to maintain about 7 letters in memory. The response accuracy of the
488 participants decreased from load4 (mean ± S.D.: 97.89% ± 1.90%) to load6
489 (91.03% ± 5.49%) and to load8 (85.49% ± 6.11%) (repeated-measures analysis
490 of variance (ANOVA), $F(2,24) = 36.55$, $p < 0.001$, **Fig. 1B**). This finding indicates that
491 the behavioral performance of participants was modulated by WM load. The response
492 accuracy for each participant is listed in **Table 1**. We recorded local field potentials
493 from depth electrodes implanted in the hippocampus and the EC (**Fig. 1C**) while
494 participants performed the task. Across all participants, 87 channels in the
495 hippocampus and 46 channels in the EC were included in the subsequent analysis (see
496 the details in **Methods**).

497 **Theta/alpha synchronization in the hippocampal-entorhinal circuit as a function**
498 **of WM load**

499 To explore the potential interaction between the hippocampus and the EC during WM
500 maintenance, we employed phase locking values (PLVs) to assess the coherence of
501 phase relationships among each channel pair connecting the two regions. The PLV up
502 to 100 Hz was computed in the time-frequency domain to reveal the dynamic
503 fluctuations of the functional connectivity. As shown in **Fig.2A**, the phase
504 synchronization up to 12 Hz (permutation test, $p < 0.05$) was found significantly

between the hippocampus and the EC throughout the entire maintenance period regardless of WM load. And this finding was confirmed by the spectral PLV across the time domain, which the real phase synchronization of hippocampal-EC was existed in the theta/alpha band (2-12 Hz; **Fig.2B**, gray area) that exceeded the threshold from the permutation test on the PLV with trials of load8 (**Fig.2B**, black line). To examine whether the theta/alpha PLV was increased with WM load increase, we made a cluster-based permutation test on the theta/alpha PLV between two load conditions for the time-frequency space across participants. As presented in **Fig.2C**, the theta/alpha PLV in the high load condition (load6/8) was higher relative to the low load condition (load4) during maintenance (cluster-based permutation, $p < 0.05$), which demonstrated consistent frequency effects during maintenance. To confirm whether the theta/alpha PLV was modulated by WM load, we separately calculated the theta/alpha PLV for load 4, load 6 and load 8 and compared the PLV between loads using a permutation test with FDR correction. Results also indicated that the theta/alpha PLV was larger in the higher load conditions than in load4 (FDR corrected: load4 vs load6, $p = 0.0042$; load4 vs load8, $p = 0.006$; load6 vs load8, $p = 0.15$; **Fig.2D**).

Next, we investigated whether inter-regional phase synchronization predicts WM load. Support vector machine (SVM) classifiers show good generalization performance for high dimensional data and have been widely used for classifying

scalp EEGs (Kumar and Gupta, 2021) and have recently been successfully used for classifying magnetoencephalography signals (Mamashli et al., 2021). Hence, we used a linear SVM classifier here to decode WM load (load 4, load 6 or load 8) on the participant level with theta/alpha PLV as features (**Fig. 1D**). Previous studies suggested that leave-one-out cross-validation (LOOCV) is applicable to obtain a reliable accuracy estimate for a classification algorithm when the number of sample in a data set is small (Wong, 2015). Thus, we applied LOOCV by splitting the data set of all participants (N = 13) from WM load into a training set of N-1 participants and a testing set of the remaining one participant, and then replicated this procedure by N times. An average decoding accuracy was obtained across all cross-validations (N times) for the classification of WM load. The statistical significance of the classification accuracy was determined by comparing the original accuracy with a null distribution created by using a randomized classifier by permuting the labels 1000 times (see details in **Methods**). As shown in **Fig.2E**, decoding accuracy using the theta/alpha PLV features for WM load (41.03% ± 4.05%) was significantly above chance level (permutation test against scrambled labels, $p < 0.05$). These results suggest that the theta/alpha PLV between the hippocampus and the EC can predict WM load for individual participants.

Directional information transfer from the hippocampus to the EC carries information on WM load

To further examine the functional relevance of directionality in the hippocampal-EC synchronization, we applied a frequency-domain GC analysis to quantify the inter-regional directional influence. We separately computed the spectral GC index in the theta/alpha band for trials with load4, load6 and load8 between the hippocampus and the EC during maintenance. Then, we examined the association between WM load and the information flow from the hippocampus to the EC and from the opposite direction, separately, using the permutation test with FDR correction. As presented in **Fig. 3A**, the GC index from the hippocampus was larger in load8 condition than in load4 (FDR corrected: load4 vs load6, $p = 0.056$; load4 vs load8, $p = 0.012$; load6 vs load8, $p = 0.30$). While there was no load effect on information transfer for the opposite direction (all $ps > 0.05$). Moreover, no significant differences in information flow between the hippocampus and EC were observed for all load conditions (paired *t*-tests, all $ps > 0.05$).

We next investigated whether the directional information flow between the hippocampus and the EC could predict WM load. The GC index in the theta/alpha band from both directions were calculated as features to decode the WM load. As shown in **Fig. 3B**, WM load could be decoded by using the GC features from the hippocampus to the EC (43.59% ± 5.83%; permutation test against scrambled labels,

565 $p < 0.05$) but not in the opposite direction (41.03% ± 5.54%; $p > 0.05$). These results
566 provide evidence at the level of individual participants that WM load affected the
567 theta/alpha directional information transfer from the hippocampus to the EC.

568

569 Given that the GC analysis is sensitive to the signal-to-noise ratio across frequency
570 bands (Cohen, 2014), we confirmed the directionality between the hippocampus and
571 the EC by calculating the phase slope index (PSI) (Nolte et al., 2008) in the
572 theta/alpha band (2-12 Hz). **Fig.3C** presents the *z*-scored PSI in the theta/alpha band
573 for the load 4, load 6 and load 8 conditions. The z-scored PSI differed between the
574 low (load4) and high loads (load6/load8). In particular, the hippocampus-driven
575 information flow was larger in load6 and load8 than the load4 condition (permutation
576 test: load4 vs load6, $z = 2.29$, $p = 0.022$; load4 vs load8, $z = 3.02$, $p = 0.0025$; FDR
577 corrected). These results confirm the findings from the GC analysis. Together they
578 indicate that the hippocampus-driven information transfer carries the information of
579 WM load.

580 **Cross-regional phase-amplitude coupling within the hippocampal-entorhinal**
581 **circuit predicts WM load**

582 Cross-regional PAC serves as a mechanism for organizing brain activity across
583 regions. Therefore, we performed cross-regional PAC in both phase-amplitude
584 combinations (low-frequency phase from the hippocampus and high-frequency

amplitude from the EC, and vice versa) using a modulation index (MI) (Tort et al., 2010; Vaz et al., 2017). To remove PAC expected by chance, the raw PAC was *z*-scored against surrogate distributions across channel pairs and participants on a population level (see Methods for details), as previous studies did (Solomon et al., 2019). As presented in **Fig.4A**, there was evident zPAC ($|z| > 1.96$) between the theta/alpha phase of the hippocampus and the gamma amplitude of the EC for each load, while no significant coupling was found in the opposite direction ($|z| < 1.96$, **Fig.4B**). Thus, we extracted hippocampal theta/alpha phase – EC gamma amplitude zPAC for further analyses.

To examine the association between cross-regional *z*PAC and WM load, we compared theta/alpha-gamma *z*PAC under different load conditions. Results showed stronger hippocampal theta/alpha phase – EC gamma amplitude *z*PAC in the high load condition (load6/8) compared to load4 (permutation test: load4 vs load6, $p = 0.047$; load4 vs load8, $p = 0.046$; load6 vs load8, $p = 0.61$; **Fig.4C**). Given the significant theta/alpha PLV findings, we added theta/alpha PLV as a regressor to examine whether it contributed to the functional effect of hippocampal theta/alpha phase – EC gamma amplitude zPAC, as previous study did (Wang et al., 2021). Our analyses revealed that the effect of WM load on *z*PAC was still significant (linear mixed-effects model: $p = 0.011$, $t = 2.71$), when controlling for the PLV ($p = 0.16$). Our

findings indicated that the load effect on hippocampal theta/alpha phase – EC gamma amplitude *z*PAC could not be explained by PLV differences.

Additionally, we fed the hippocampal theta/alpha phase – EC gamma amplitude *z*PAC features into the linear SVM classifier to decode the WM load. We found that the decoding accuracy using cross-regional *z*PAC with the theta/alpha phase of the hippocampus modulating the gamma amplitude of the EC reached a significant level (51.28% ± 8.12%; permutation test against scrambled labels, $p < 0.05$, **Fig.4D**). These results are in line with the univariate analysis.

Effect of WM load on inter-regional interaction between the hippocampal subregion and EC

The hippocampus, a complex structure, comprises anterior and posterior subregions that exhibit distinct function during WM maintenance (Li et al., 2022). Consequently, we performed separate analyses for the anterior hippocampus-EC and posterior hippocampus-EC connections. Utilizing permutation tests with FDR correction, we compared the metrics of PLV, GC, and PAC between different WM loads. Regarding the theta/alpha PLV between the anterior hippocampus and the EC, we found a significantly higher PLV in load 8 compared to load 4/6 (FDR corrected: load4 vs load8, p = 0.036; load6 vs load8, p = 0.045; **Fig.5A** top). For the theta/alpha PLV between the posterior hippocampus and the EC, we observed a higher PLV in load 6/8 than load 4 (FDR corrected: load4 vs load6, $p = 0.007$; load4 vs load8, $p = 0.007$;

load6 vs load8, $p = 0.86$; **Fig.5A** bottom). Regarding the theta/alpha GC index between the anterior hippocampus and the EC, we found a higher GC value from the anterior hippocampus to EC in the high load conditions compared to the low load condition (FDR corrected: load4 vs load6, $p = 0.076$; load4 vs load8, $p = 0.044$; load6 vs load8, $p = 0.43$; **Fig.5B** top). However, in the opposite direction, there were no significant differences observed (permutation test, all *ps* > 0.05; **Fig.5B** bottom). Regarding to the theta/alpha GC index between the posterior hippocampus and the EC, we did not find any difference between loads in either direction (permutation test, all *ps* > 0.05; **Fig.5C**). For the anterior hippocampal theta/alpha – EC gamma zPAC, no significant differences between loads were found (permutation test, all *ps* > 0.05; **Fig.5D** top); for the posterior hippocampal theta/alpha – EC gamma zPAC, stronger coupling in high load condition was found relative to low load condition (FDR corrected, load4 vs load6, $p = 0.026$; load4 vs load8, $p = 0.047$; load6 vs load8, $p = 0.56$; **Fig.5D** bottom). In addition, we also directly compared measurements from the two directions under each load condition using paired *t*-tests, leading to no directional difference in any comparison (all *ps* > 0.05). In summary, our observations indicate that WM load affects both the anterior and posterior hippocampus in a comparable manner, which is consistent with the impact of WM load on the connections between the entire hippocampus and the EC.

Local Power Analysis for WM Load

The above analyses revealed that the WM load can be decoded by the hippocampal-EC interactions in the theta/alpha band and in the theta/alpha-gamma coupling. We next asked whether local activity in the hippocampus and the EC indicate WM load. We calculated the time-frequency power for each channel separately for trials with load4, 6 and 8. The power outputs were *z*-scored against the pretrial baseline distributions to assess the significance of the task-induced power effects per trial (see **Methods**). We separately calculated the *z*-scored power within the hippocampus and EC in the theta/alpha band for each load in each participant and fed the power features into the SVM classifier to decode the WM load with LOOCV. As shown in **Fig.6A**, no significant results were found for any of the regions for the classification of WM load (Hipp: 41.03% ± 5.54%; EC: 46.15% ± 6.01%; permutation test against scrambled labels, $p > 0.05$). This analysis indicated that the load effects on hippocampal-entorhinal interaction were not significantly explained by load effect on the power at hippocampal or entorhinal channels. We would like to stress that these results do not exclude a role of local activity.

As previous studies noted (Donoghue and Watrous, 2023), the conventional analytical approaches concerning neural oscillatory activity have a tendency to conflate periodic and aperiodic activities. To test whether the impact of aperiodic activity could explain our observations of functional effects, we first extracted the aperiodic activity using

FOOOF toolbox (Donoghue et al., 2020b) from the hippocampus and EC for each load and participant and then conducted a repeated-measures ANOVA with a within-group effect of load across the frequency spectrum. Results indicated that the aperiodic activity did not exhibit significant differences among WM loads (all *ps* > 0.05, **Fig. 6B**). Next, we added the aperiodic activity as a regressor in the analysis of functional effects of hippocampal theta/alpha phase – EC gamma amplitude zPAC (see Methods for details). The load was still significant to zPAC (linear mixed-effects model, $p = 0.011$, $t = 2.71$), even when controlling for the aperiodic activity of the hippocampus in the theta/alpha range ($p = 0.43$) and of the EC in the gamma range ($p = 0.07$). This result validated our previous findings that the modulation of the hippocampal theta/alpha phase on the EC gamma amplitude carries the load information. To further rule out the bias of aperiodic activity on the information flow between the hippocampus and the EC, we did similar analysis for GC index (see Methods for details). Our finding noted that the effect of load was significantly associated with hippocampal driven GC (linear mixed-effects model, $p = 0.024$, $t = 2.82$), while neither the aperiodic activity from the hippocampus ($p = 0.35$) nor those from the EC ($p = 0.54$) significantly contributed to it. Then, we did a similar analysis with EC driven GC as dependent variable, none of the effects, including load and aperiodic activity in either region, reached statistical significance (all *ps* > 0.05). This result replicates our previous findings and underscores the influence of WM load on hippocampal driven GC, even when controlling for aperiodic components.

In summary, WM maintenance was accompanied with elevated synchrony within the hippocampal-entorhinal interaction, with a theta/alpha coordinated hippocampal-driven influence on the EC. This influence, including information transfer from the hippocampus to the EC in theta/alpha band and the hippocampal theta/alpha phase entraining EC gamma amplitude, increased from WM low load (load4) to high load conditions (load6/8), and predicted WM load (**Fig.6C**).

Discussion

We showed that WM maintenance is associated with coordinated neural oscillations between the hippocampus and the EC in specific oscillatory modes of frequency and direction. In particular, we observed increased hippocampal driven information transfer via the theta/alpha band, and increased PAC between the theta/alpha phase of the hippocampus entraining the gamma amplitude of the EC. This inter-regional communication during maintenance increased with WM load and predicted WM load. These findings provided direct neural evidence of hippocampal-EC interactions during WM maintenance in humans and links a specific inter-regional activity pattern to WM load.

The interregional oscillatory dynamics are consistent with known structural and functional connections between the hippocampus and the EC. Anatomical studies found that the EC sends projections to and receives monosynaptic input from the hippocampus (van Groen et al., 2003; Small et al., 2011). Optogenetic inhibition of this circuit in mice resulted reduction in both inter-regional connectivity and the correct execution of WM-guided behavior (Yamamoto et al., 2014). Our results are thus consistent with animal literature suggesting the contribution of hippocampal-EC communication to WM processing and extended these findings to humans.

To investigate this communication, we computed the hippocampal-EC phase synchronization, a neural mechanism that is thought to enhance neural communication and plasticity (Fell and Axmacher, 2011; Daume et al., 2023). Consistent with this notion, previous studies have shown that theta/alpha band phase synchronization facilitates the recruitment of WM-related regions, including various cortical areas (Johnson et al., 2018b) as well as the hippocampus and cortical areas (Boran et al., 2019; Dimakopoulos et al., 2022), thereby supporting WM function.

To date, only a handful of human studies have collected direct intracranial data on both the hippocampus and the EC during WM processing, and all looked at each region separately rather than at their connectivity (Kornblith et al., 2017; Boran et al., 2019; Boran et al., 2022). Considering the evidence for decoding WM load through

728 inter-regional interaction in the present study, our findings point to a coordinated role
729 of the hippocampus and the EC in WM information maintenance. These results
730 underscore the significance of investigating connections to understand the neural
731 mechanisms of WM. Our results align with animal findings mapping the non-spatial
732 dimension of the hippocampal-EC circuit (Aronov et al., 2017). They together suggest
733 a common circuit mechanism that contribute to diverse behavioral tasks and
734 supporting cognitive processes beyond spatial navigation.

735

736 Both the GC and PSI measures, despite being based on different principles
737 (magnitude and phase), consistently demonstrated that the net information flow is
738 from the hippocampus to the EC during WM maintenance. This is in agreement with
739 animal results where the hippocampus receives sensory information from the EC
740 during encoding and subsequently processes and returns memory-related information
741 (Buzsáki and Tingley, 2018; Rozov et al., 2020). The hippocampal outflow during
742 WM maintenance may also contribute to the transfer of memories from short-term
743 storage in the hippocampus to long-term storage in the neocortex as part of the
744 memory consolidation process (Frankland and Bontempi, 2005; Kaminski and
745 Rutishauser, 2020). These findings provide further confirmation of the directional
746 modulation observed in humans and point to a role of neural oscillations in regulating
747 this modulation. Recent studies found that memory consolidation may start as early as
748 at the end of encoding (Ben-Yakov et al., 2013; Zhang et al., 2021). In agreement

749 with this, hippocampal outflow during the post-encoding period can decode
750 subsequent memory performance (Zhang et al., 2021), and WM maintenance
751 contributes to long-term memory performance (Ranganath et al., 2005; Kaminski and
752 Rutishauser, 2020). Taken together, our results may have implications in
753 understanding long-term memory consolidation.

754

755 The theta/alpha oscillations drive inter-regional communication in the hippocampal-
756 EC circuit during WM maintenance. Previous studies reported theta/alpha frequency
757 synchronization between the hippocampus and cortical regions (Johnson et al., 2018b;
758 Dimakopoulos et al., 2022) and between cortical regions (Johnson et al., 2018a)
759 during WM maintenance. Computational models have suggested that these
760 oscillations coordinate the proper timing of interactions between the hippocampus and
761 the EC (Kurikawa et al., 2021). Here, we speculate that, during WM maintenance,
762 task-relevant mnemonic signals are strengthened by theta connectivity, and stronger
763 distracting signals are suppressed by higher levels of alpha synchronization. Rodent
764 studies showed that these oscillations in the hippocampal-EC circuit facilitate synaptic
765 plasticity (Diana et al., 2007; Buzsaki and Moser, 2013; Colgin, 2013). Human iEEG
766 studies reported that the hippocampal-EC communications via the theta band
767 contributed to episodic memory (Solomon et al., 2019). We extend these findings to
768 WM by reporting a load-dependent increase in the hippocampal-EC connectivity.

769

In subsequent analysis involving cross-frequency coupling features, we observed that WM load was associated with theta/alpha-gamma phase-amplitude couplings (PACs) in the hippocampal-EC circuit. Previous studies have consistently highlighted the involvement of rhythmic activity at theta, alpha, and gamma frequencies in WM maintenance (Bragin et al., 1995; Sarnthein et al., 1997; Axmacher et al., 2007; Michels et al., 2008; Roux and Uhlhaas, 2014; Daume et al., 2023). Theta-gamma PAC has been proposed to modulate synaptic plasticity (Huerta and Lisman, 1995) and organize complex mnemonic information (Heusser et al., 2016), while alpha-gamma PAC has been implicated in the gating of sensory information and read-out of relevant WM items (Roux et al., 2013; Davoudi et al., 2021). Cross-regional PAC describes coordinated brain activity between brain regions. However, it is important to note that the presence of cross-regional PAC does not imply directional causality. Canolty and Knight (2010) introduced a model explaining how synchronized theta oscillations and local PAC regulate cortical activity in relation to the hippocampus, suggesting that inter-regional cross-regional PAC is a secondary outcome of this cortical organization. Drawing upon the functional roles of local theta/alpha-gamma PACs mentioned above, we hypothesize that cross-regional PAC may serve as a mechanism for the formation of an integrated memory representation through precise coordination of local high-frequency oscillations. Besides, coupling between hippocampal theta phase and gamma activity in the EC, in the same frequency band and direction as found in our study, was suggested supporting episodic memory (Wang et al., 2021). Our study extends this finding to WM and they together imply

792 the cross-regional PAC as a key neurophysiological mechanism in mnemonic
793 processing.

794

795 Besides, we found distinctions in inter-regional interaction patterns between low and
796 high WM load conditions. This pattern aligns with our earlier research (Boran et al.,
797 2019), which demonstrated that load-sensitive maintenance neurons in the
798 hippocampus exhibited a plateauing effect at high-load levels rather than showing
799 incremental increases of firing rates with WM load. However, we do not interpret this
800 as reflecting a binary relationship between inter-regional connectivity and WM load.
801 Instead, this observation may suggest the presence of processing capacity limits (von
802 Allmen et al., 2013), which are closely tied to the concept of workload. We reported
803 that the averaged capacity was 7.2 (see Results), indicating that participants were
804 capable of maintaining at least seven letters in memory. However, when attempting to
805 maintain 8 letters, they may reach or exceed their capacity limits. Consequently, we
806 might not observe further elevation in inter-regional connectivity.

807

808 In summary, our results provide direct evidence that WM maintenance is supported
809 by the unidirectional influence from the hippocampus to the EC via the theta/alpha
810 band in a load-dependent manner. We have extended previous knowledge of the
811 contribution of the hippocampal-EC circuit on WM in animals to humans.

Data availability

The data set was analyzed and described earlier (Boran et al., 2019; Boran et al., 2020; Dimakopoulos et al., 2022; Li et al., 2022; Li et al., 2023a) and is freely available for download at https://doi.gin.g-node.org/10.12751/g-node.d76994/. The task is freely available for download at http://www.neurobs.com/ex_files/expt_view?id=266. Links to updates and further data sets can be found at https://hfozuri.ch.

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Table 1 Subject characteristics

Participant	Age	Sex	ECsites	Hipp sites	Response accuracy % for load4	Response accuracy % for load6	Response accuracy % for load8	Number of trials (sessions)

1	24	Female	2	6	100	92	85	200 (4)
2	39	Male	4	8	96	81	79	339 (7)
3	18	Female	2	6	98	94	89	147 (3)
4	28	Male	4	8	100	94	89	99 (2)
5	31	Male	4	8	99	91	91	349 (6)
6	47	Male	4	8	99	92	93	197 (4)
7	56	Female	4	7	97	96	75	249 (5)
8	19	Female	4	8	100	94	85	96 (2)
9	35	Male	4	6	99	92	87	199 (4)
10	51	Female	4	6	96	91	86	382 (8)
11	30	Male	4	4	98	88	78	200 (4)
12	29	Female	2	4	98	100	95	295 (6)

13	56	Male	4	8	94	80	80	295 (6)

Figure legends

Fig. 1 Working memory task, recording sites, and schematic of multivariate pattern analysis.

(A) An example trial of the task. Each trial consisted of a set of consonants, including four, six or eight letters for 2 s (encoding). The number of letters was thus specific for the memory load (load 4, load 6 and load 8). After a delay for 3 s (maintenance, red), a probe letter was shown, and the participants indicated whether the probe was or was not contained in the stimulus letter set.

(B) Response accuracy decreased with load 4 (light red), 6 (light green) and 8 (light blue) across the group of participants. ** p <0.01.

(C) Channel locations of all participants in MNI152 space. Recording regions are indicated by different colors (red, hippocampus; blue, EC).

(D) Schematic of the multivariate pattern analysis. Phase locking value (PLV), Granger causality index (GC), and phase-amplitude coupling (PAC) were calculated separately between the Hipp (red) and the EC (blue) for trials with WM load 4, load 6 and load 8. The patterns of PLV (e.g., theta/alpha PLV), GC (e.g., theta/alpha GC) and PAC (e.g., theta/alpha-gamma PAC) were separately used to train the support vector machine (SVM) classifier to classify the WM load (load 4, load 6 or load 8). Specifically, we converted the features to a feature vector (M), fed them into a linear SVM classifier, trained the classifier on the data from N-1 participants (N-1 × M), and tested it on the remaining one participant (1 × M). We used inter-participant cross validation by leaving one participant out for validation and replicated the classification. The accuracy was used as the performance metric.

Fig. 2 Frequency-specific hippocampus-EC phase locking value (PLV) during maintenance is load sensitive.

(A) Phase synchrony (PLV) between the hippocampus and the EC was identified across all participants for the load 4 (left), load 6 (middle) and load 8 (right) conditions, with greater low-frequency synchrony during maintenance of WM load. The PLV ranged from 0 to 1, with warmer colors indicating higher PLV. The PLV maps show the PLV up to 12 Hz that survived the threshold from trials with load8 at $p < 0.05$ (permutation test).

(B) Spectral PLV across the time domain within 1-100 Hz between the hippocampus and the EC across participants for the load 4 (light red), load 6 (light green) and load 8 (light blue) conditions (SEM shown as shaded area around the mean trace) with peaks in the theta/alpha range (2-12 Hz, light gray area) used for subsequent analyses. Black line indicates the threshold from the permutation test on the PLV with trials of load8.

(C) Significant increase in the PLV with trials of load6 (left panel) and load8 (right panel) related to the PLV with trials of load4 in the theta/alpha (2-12 Hz) frequency range during maintenance (cluster-based permutation, $p<0.05$). Warmer color denotes the work-load increase in the time-frequency space of PLV.

(D) Theta/alpha PLV of the hippcampus-EC across all participants in load 4, load 6 and load 8. The synchronization was larger in higher loads than in load 4. ** $p < 0.01$.

(E) Decoding accuracy based on the theta/alpha PLV features between the hippocampus and the EC. The left panel shows the null distribution of the statistic for the decoding analysis, which were created using classifiers with randomized training labels. The threshold of significance is marked with a vertical dashed line ($p < 0.05$). The right panel shows the decoding accuracy for WM load by using the theta/alpha PLV, the accuracy was above the threshold (horizontal dashed line, labeled with asterisk, * $p < 0.05$).

Fig. 3 Information flow between the hippocampus and the EC reflects WM load.

(A) Averaged GC index in the theta/alpha band from the Hippocampus to the EC (left) as well as from the opposite direction (right) across all participants for load4 (light red), load6 (light green), and load8 (light blue). Higher WM load was associated with increased GC from the hippocampus to EC, but not in the opposite direction. * $p < 0.05$, # $0.05 < p < 0.1$.

(B) Decoding accuracy using the GC features from the hippocampus to the EC (red) and from the opposite direction (blue), respectively. The left panel shows the null distribution of the maximum statistics for theses decoding analyses, which were created using classifiers with randomized training labels. The threshold of significance is marked with a vertical dashed line ($p < 0.05$). The right panel shows the decoding accuracy using the theta/alpha GC index from both directions. WM load could be decoded by using the GC features from the hippocampus to the EC but not in the opposite direction. * $p < 0.05$.

(C) The *z*-scored phase slope index (PSI) in the theta/alpha band across all participants for the load 4, load 6 and load 8 conditions. Positive values indicate that the hippocampus leads the EC. The *z*-scored PSI in the high load conditions (load 6/8) is higher than the load 4 condition. ** $p < 0.01$, * $p < 0.05$.

Fig. 4 Cross-regional coupling between the hippocampus and the EC.

(A) Average *z*-scored PAC between hippocampal phase and EC amplitude, with logarithmic frequency scaling on both phase and amplitude axes across participants for trials with load4, load6, and load8. Notably, there is conspicuous cross-regional z-scored PAC between hippocampal theta/alpha (2-12 Hz) phase and EC gamma (30-100 Hz) amplitude ($|z| > 1.96$, highlighted in red).

(B) *z*-scored PAC depicting EC phase - hippocampus amplitude coupling for trials with load4, load6, and load8. In the theta/alpha (2-12 Hz) phase and gamma (30-100 Hz) frequency range, no significant coupling is evident ($|z| < 1.96$, represented in white).

(C) Averaged theta/alpha-gamma zPAC across participants from both directions in load4 (light red), load6 (light green) and load8 (light blue) conditions. The coupling between the hippocampus theta/alpha phase and the EC gamma amplitude was larger in the high load conditions (load6/8) than the low load condition (load4). * $p < 0.05$.

(D) Decoding accuracy for WM load by using the zPAC features from hippocampal theta/alpha phase and EC gamma amplitude. The decoding accuracy (red, right panel) reached a significant level (horizontal dashed line in the right panel), which is 95^{th} percentile of the null distribution of decoding accuracy (left panel).

Fig.5 Inter-regional connectivity between the anterior/posterior hippocampal subregion and EC.

(A) Averaged theta/alpha PLV between the anterior (top panel)/posterior hippocampus (bottom panel) and the EC for trials with load4 (light red), load6 (light green) and load8 (light blue). ** $p < 0.01$, * $p < 0.05$.

(B) GC value between the anterior hippocampus and the EC. Top panel: the GC value from the anterior hippocampus to the EC was higher in the high load condition (load6/8, light green/blue) than the low load condition (load4, light red). Bottom panel: the GC value from the EC to the anterior hippocampus did not show any significant differences between WM loads. * $p < 0.05$, # $0.05 < p < 0.1$.

(C) GC values between the posterior hippocampus and the EC. Both the GC value from the posterior hippocampus to the EC (top panel) and the GC value from the EC (bottom panel) to the posterior hippocampus did not display significant differences across working memory loads.

(D) The *z*PAC values with the theta/alpha phase of the anterior hippocampus modulating the gamma amplitude of the EC (top panel) did not show any differences between loads. While the *z*PAC values with the theta/alpha phase of the posterior hippocampus modulating the gamma amplitude of the EC (bottom panel) was higher in high load conditions (load6/8, light green/blue) compared to the load 4 condition (load4, light red). * $p < 0.05$.

Fig. 6 Hippocampal-entorhinal communications reflects WM load.

(A) Decoding accuracy for WM load by using the theta/alpha power features within the hippocampus (red) and the EC (blue), respectively. The decoding accuracy in neither region exceeds the threshold of significance (dashed line, $p < 0.05$).

(B) Aperiodic activity across the frequency spectrum from the hippocampus (left) and the EC (right). No difference was found among load4 (blue), load6 (green) and load8 (red). SEM shown as shaded area around the mean trace.

(C) Schematic of the hippocampal-entorhinal communications for low (top) and high (bottom) WM load. Unidirectional hippocampal influence on the EC increased with WM load via the theta/alpha band.