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Supplemental Information

Theta Phase-Coordinated Memory Reactivation Reoccurs in a Slow-Oscillatory Rhythm during NREM Sleep

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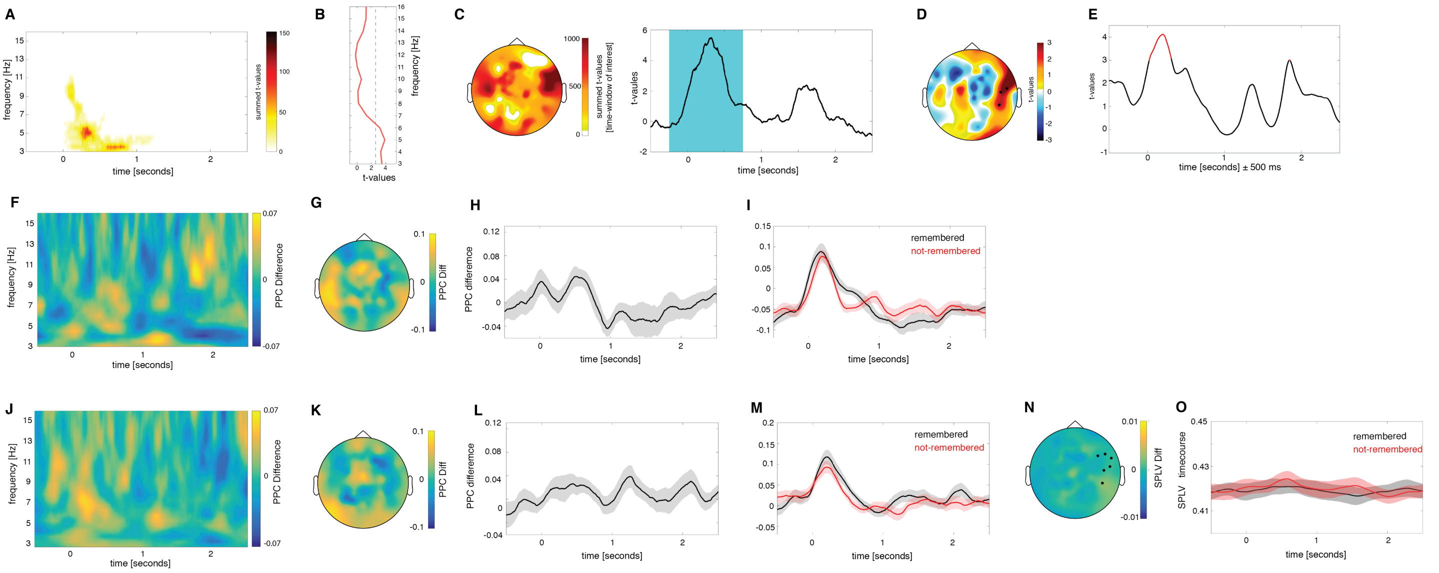

Figure S1 related to Figure 2+3. Assessing content-specificity. (A-E) To evaluate whether phase similarity in the theta range during recall mirrors content specificity, phase similarity was estimated for successfully retrieving the same word in consecutive retrieval instances (i.e. similarity [$word_{a, recall1}$, $word_{a, recall2}$; $word_{b, recall1}$, $word_{b, recall2}$; ...]) and contrasted against phase similarity for successfully retrieving different words in consecutive retrieval instances (similarity [$word_{a, recall1}$, $word_{b, recall2}$; $word_{c, recall1}$, $word_{d, recall2}$; ...]). **(A)** We found significantly higher phase similarity when retrieving the same as compared to differing memory content in the theta range (cluster-randomization: P = 0.002, corrected for multiple comparisons), **(B)** peaking at 5 Hz. **(C)** The time-course of the phase similarity at 5 Hz displayed an early significant difference between remembered and non-remembered words (cluster-randomization: P = 0.008, corrected for multiple comparisons). The topography shows summed t-values of the averaged difference at 5 Hz between 0 and 2.5 seconds. These results support the content-specificity of theta phase similarity during recall. **(D+E)** To estimate the content specificity between recall and TMR, phase similarity was estimated for successfully remembered items of the same content (i.e. similarity [$word_{a, recall2}$, $word_{a, TMR}$; $word_{b, recall2}$, $word_{b, TMR}$; ...]) and contrasted against phase similarity for random pairs of remembered items, thus with differing memory content (i.e. similarity [$word_{a, recall2}$, $word_{b, TMR}$; $word_{c, recall2}$, $word_{d, TMR}$; ...]) . **(D)** Test statistics on the averaged difference between same and different memory content revealed the reactivation of recall-related phase-patterns at 5 Hz during TMR during sleep (cluster-randomization: P = 0.04, corrected for multiple comparisons) over right temporal electrodes. **(E)** To examine the time-course, t-values were averaged across significant electrodes (n = 6) and t-statistics were computed for every time-point. Two distinct reactivation episodes emerged, peaking at 230ms (t_{16}= 4.06, P = 0.001) and 1800ms (t_{16} = 3.01, P = 0.008). These results further support the content-specificity of theta phase similarity between wakefulness and sleep. **Encoding / Recall phase similarity (unmasked data; (F-M)).** PPC difference between remembered/non-remembered words for the same stimuli during **(F)** encoding and recall1, indicates that similarity measures were not driven by similarities in auditory stimulation **(G)** topography of the phase similarity at 5 Hz **(H)** time-course of phase similarity at 5 Hz (remembered minus not-remembered conditions averaged across the 83 electrodes which showed the content-specific phase similarity effect in the main analysis). **(I)** individual time-courses for phase similarity at 5 Hz for remembered and not-remembered words (again averaged across the 83 electrodes). PPC difference between remembered/non-remembered words for the same stimuli during **(J)** encoding and recall2, indicate that similarity measures were not driven by similarities in auditory stimulation **(K)** topography of the phase similarity at 5 **(L)** time-course of phase similarity at 5 Hz (remembered minus not-remembered conditions, averaged across the 83 electrodes which showed the content-specific phase similarity effect in the main analysis). **(M)** individual time-courses for phase similarity at 5 Hz for remembered and not-remembered words (again averaged across the 83 electrodes). **Encoding / TMR phase similarity (unmasked data; (N+O)).** S-PLV measures contrasted between remembered/non-remembered words for the same stimuli during encoding and TMR at 5 Hz **(N)** topography of phase similarity at 5 Hz indicating no difference between conditions **(O)** time-course of phase similarity at 5 Hz. Shading denotes SEM.

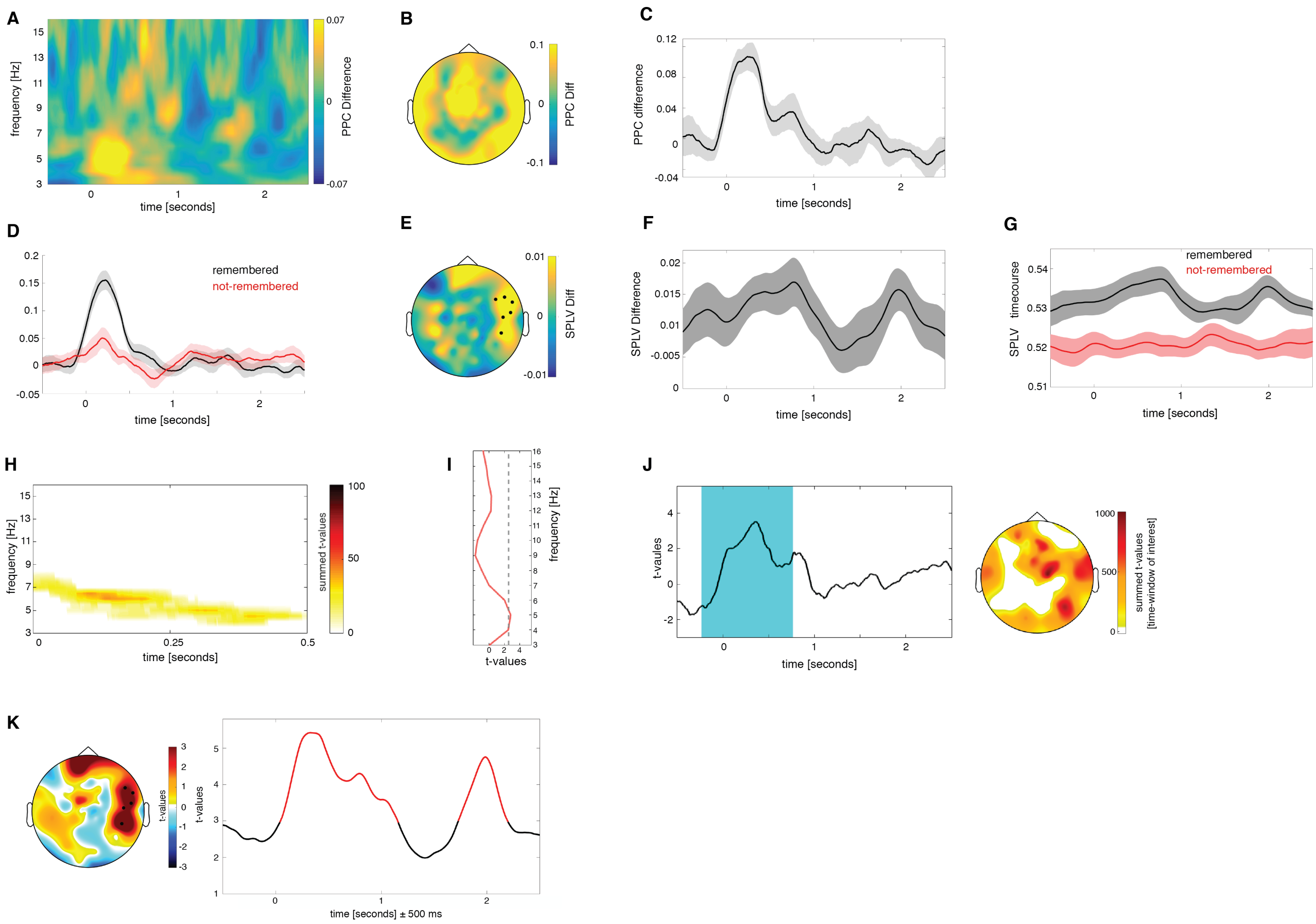

Figure S2 related to Figure 2+3. (A-G) Word specific phase-similarity (unmasked data). (A) PPC difference between remembered/non-remembered words for retrieving the same memory content during consecutive recall instances (recall1 / recall2), shows higher phase similarity in an early time window in the theta range. **(B)** topography of the effect at 5 Hz **(C)** time-course of the difference at 5 Hz (remembered minus not-remembered, averaged across all electrodes (n = 32) showing a significant similarity effect at 5 Hz). **(D)** individual time-courses for phase similarity at 5Hz for remembered and not-remembered words (again averaged across 32 electrodes). **(E)** S-PLV difference between remembered/non-remembered words for the same memory content during recall2 and TMR, indicate a higher difference in phase similarity at 5 Hz over right temporal electrodes. **(F)** time-course of the phase similarity difference at 5 Hz (remembered minus not-remembered conditions, averaged across electrodes highlighted in (E)). **(G)** individual time-courses for phase similarity at 5 Hz for remembered and not-remembered words, averaged across electrodes highlighted in (E). Shading denotes SEM.

(H-J) Phase-similarity during awake retrieval defined by retrieval success in recall1 and recall2 Phase similarity was computed separately per condition ($\text{similarity}_{\text{remembered}}$, $\text{similarity}_{\text{non-remembered}}$) and contrasted. Retrieval success during recall1 and recall2 determined the assignment of words to condition (as compared to the main analysis where only recall2 retrieval success determined assignment of words to condition). Please note that the overall trial number was significantly smaller as compared to our main analysis, due to the fact that in recall1 less words were remembered. Since trial numbers were matched between conditions, also a lower number of trials entered the non-remembered category. Thus, we restricted our analyses to the time-window were we found effects in the main analysis (0-500ms), while including the same frequency range (3-16 Hz). **(H)** We found significantly higher phase similarity for remembered words as compared to non-remembered words in the theta range (cluster-randomization: $P = 0.022$, corrected for multiple comparisons), **(I)** again peaking at 5 Hz (for illustration please see below). **(J)** The time-course of the phase similarity at 5 Hz displayed an early significant difference between remembered and non-remembered words (cluster-randomization: $P = 0.015$, corrected for multiple comparisons). The one second time-window around the center of the strongest cluster is highlighted. For the topography summed t-values of the averaged difference between 0 and 2.5 seconds were plotted. **Phase-similarity between wake/sleep defined by retrieval success in recall2 and TMR (K)** Phase similarity was computed separately per condition ($\text{similarity}_{\text{remembered}}$, $\text{similarity}_{\text{non-remembered}}$) and contrasted. Retrieval success during recall2 and TMR determined the assignment of words to condition (as compared to the main analysis where only retrieval success after sleep determined assignment of words to condition). Test statistics on the averaged difference between remembered / non-remembered words revealed higher phase similarity for remembered items at 5 Hz (cluster-randomization: $P = 0.016$, corrected for multiple comparisons) over right temporal electrodes. To examine the time-course of the reactivation effect, similarity-measures were again averaged across significant electrodes and t-statistics were computed for every time-point. Two distinct reactivation episodes emerged, peaking at 430ms ($t_{16}= 5.16$, $P = 0.00009$) and 2000ms ($t_{16} = 4.62$, $P = 0.0002$).

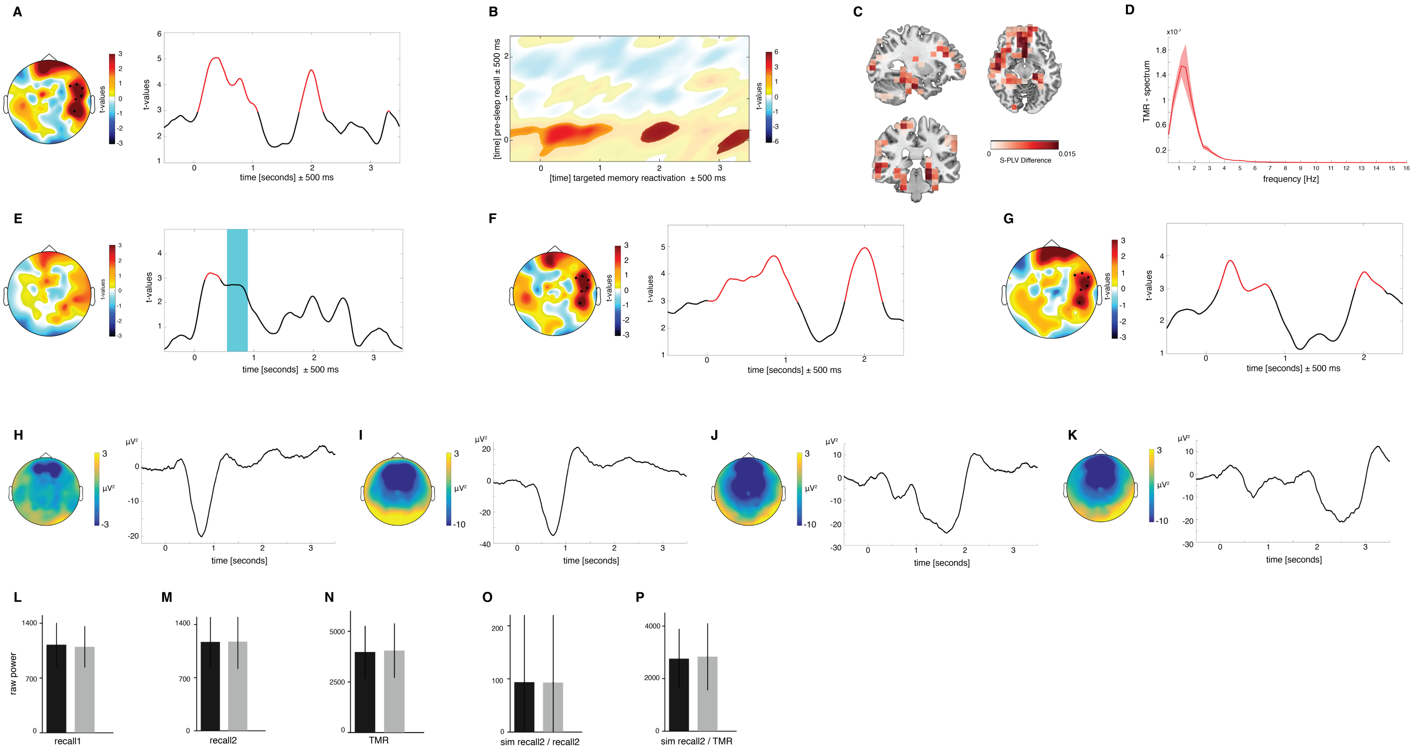

Figure S3 related to Figures 2+3. (A-E) Assessing word specific phase-similarity between recall2 and TMR up to 3.5 seconds. Due to the length of our TMR data segments and the 1 second width of the sliding time-window (from retrieval), robust similarity values could be obtained up to 2.5 seconds after cue presentation with regards to the TMR data. For any further time-point after 2.5 seconds a decreasing amount of data was available, thereby adding noise to the measurements. Still, to characterize the perpetuating effects of TMR induced reactivation-processes we investigated similarity measures up to 3.5 seconds (even though values after 2.5 seconds have to be taken with caution). **(A)** Re-current reactivation of recall-related phase-patterns at 5 Hz during TMR emerged over right temporal electrodes. The topography displays the test-statistics of the averaged difference in phase similarity between remembered and not-remembered words (0 - 3.5 seconds). The time-course depicts t-values averaged across highlighted electrodes (n = 6). The phase similarity at a given time point reflects the similarity computed in a window of +/- 500ms around this time point. **(B)** Assessing phase-similarity at 5 Hz between every time-point of retrieval and TMR confirmed the reoccurring pattern of similarity. Three distinct reactivation episodes emerged, peaking at 390ms (t_{16}= 4.49, P = 0.0003), 1990ms (t_{16} = 4.59, P = 0.0002) and 3310ms (t_{16} = 3.31, P = 0.004). **(C)** Source reconstruction (DICS beamformer) of the effects shown in (a). The difference in phase similarity for remembered and not-remembered items indicates large differences in right (para)hippocampal regions and left-frontal areas. **(D)** Frequency spectrum of the TMR similarity measures showed a ~1 Hz periodicity of reactivation processes. Shading denotes SEM **(E)** In line with behavioral predictions, providing a target stimulus after the TMR-cue blocked associated reactivation processes. The time course depicts t-values averaged across highlighted electrodes in (a). Presentation of the target word is highlighted in petrol. Only a brief reactivation effect at 270ms (before target word onset) emerged. The topography displays the test-statistics of the averaged difference in phase similarity between remembered and not-remembered words (0 - 3.5 seconds). No significant cluster was found. **(F+G) Varying the sliding time window width. (F)** Using a sliding time-window containing the temporal pattern from 'recall 2' with a width of 800 ms (4 cycles) to obtain phase similarity between recall and TMR indicates a highly comparable results pattern as obtained in the main analysis, with a re-current reactivation of recall-related phase-patterns at 5 Hz during TMR emerging over right temporal electrodes. The topography exhibits the test-statistics of the averaged difference in phase similarity between remembered and not-remembered words (0 - 2.5 seconds). To examine the time-course, similarity-measures were averaged across significant electrodes (n = 6) and t-statistics were computed for every time-point. Two reactivation episodes emerged, peaking at 850 ms (t_{16}= 4.66, P = 0.0002) and 2010 ms (t_{16}= 4.96, P = 0.0001). **(G)** Using a sliding time-window with a width of 1200 ms (6 cycles) to obtain phase similarity between recall and TMR also indicates a re-current reactivation of recall-related phase-patterns at 5 Hz during TMR emerging over right temporal electrodes (the reduced robustness of the effects might indicate that the usage of a rather long sliding time-window comes at cost of specificity). The topography exhibits the test-statistics of the averaged difference in phase similarity between remembered and not-remembered words (0 – 2.5 seconds). To examine the time-course, similarity-measures were averaged across significant electrodes (n = 6) and t-statistics were computed for every time-point. Two reactivation episodes emerged, peaking at 740 ms (t_{16}= 3.13, P = 0.0064) and 2010 ms (t_{16}= 3.5, P = 0.0029). **(H-K) Slow oscillation detection. (H)** Mean EEG signal of all detected SOs (at Fz) averaged (across subjects) time locked to cue presentation (t = 0 s). **(I)** Mean EEG signal of detected SOs during the first second (n = 168.29 ± 51.58) **(j)** second (n = 118.35 ± 39.49) and **(K)** third second (n = 118.35 ± 39.49 after stimulus onset (t = 0 s). A repeated measures ANOVA indicated a significant difference between the number of detected SOs for the three time segments ($F_{(2,32)}$= 31.26, P < 0.001). Post-hoc t-tests revealed that the number of SOs occurring in the first second was significantly higher as compared to the second time-segment (t = 5.31, P < 0.001), while the same direction was evident when comparing the second and third second (t = 3.85, P < 0.001). **(L-P) Spectral power control analyses.** There was no significant power-difference between remembered and non-remembered items with regards to **(L)** recall1 (P > 0.14) **(M)** recall2 (P > 0.15) and **(N)** TMR (P > 0.35; all results were controlled for multiple comparisons across space and time using cluster-based permutation statistics). We further estimated oscillatory power for the very same contrasts as utilized in the phase similarity analyses for recall1 vs. recall2 **(O)** and recall2 vs. TMR **(P)**, respectively (see Supplementary Methods for details). No significant differences were observable in both analyses (P >0.8; P >0.35; results were controlled for multiple comparisons across space and time using cluster-based permutation statistics). For illustrative purposes oscillatory power was averaged over the electrodes of interest. Note that increasing the frequency range to 3-7 Hz also yielded no significant differences for all the analyses displayed here (all p's > 0.3).

Supplemental Experimental Procedures:

Task and Procedure

German speaking participants, performed a vocabulary-learning task in the evening (~10pm). The task consisted of 120 Dutch words and their German translation. With regards to the first learning round, each trial consisted of a Dutch word, which was succeeded by the German translation. All words were presented via loudspeaker. The trials of the second round (referred to as 'recall1') started with the presentation of the Dutch word (cue), followed by a question mark for up to 7 seconds. The participants were asked to vocalize the correct German translation if possible (target). within the 7 seconds (if possible) or to indicate if they were not able to do so. In any case, the correct German translation was presented afterwards. The same cued recall procedure was accomplished in the third round ('recall2'), except that performance feedback was omitted. Participants correctly recalled 43.47 ± 2.56 out of 120 words during recall1 and 62.05 ± 3.22 (out of 120) during recall2. The learning phase was followed by a 3 hours retention interval of sleep.

During NREM sleep, subsets of the Dutch cue words learned before the retention interval were repeatedly presented for 90 minutes via loudspeaker. Cues were presented every ~4 seconds during stable NREM sleep (sleep stages N2 and SWS) via loudspeaker (50 dB sound pressure level) summing up to ~ 16 repetitions per word in random order. To assure that words were exclusively presented during NREM sleep (sleep stages N2 and SWS), sleep was permanently monitored by the experimenter. The stimulation protocol was manually interrupted whenever signs of arousals, awakenings or REM sleep were visible. Memory cues were presented either as single cues (only the Dutch words), 40 as word pair cues (Dutch and German words) and 40 were not replayed at all. During word pair cueing, the presented word-pairs consisted of correct word-pairs just as learned before the retention interval for 8 participants, while 9 participants received incorrect targets following the cues (as compared to learning). The false cue-target combinations were created by randomly intermixing the Dutch and German words of this category. Thus, new Dutch–German word combinations for presentation during sleep were formed.

Importantly opposed to replaying single cues, cueing of word pairs, irrespective of the category (correct, wrong), was associated with a suppression of the beneficial effects of cueing. Thus, we capitalized our main analysis on the single-cue TMR condition, while the word-pair condition served as control. In all three categories, the relation of remembered and non-remembered word pairs of the last learning trial before sleep was maintained. Hence, all categories comprised the same number of remembered and non-remembered words before sleep. All words were individually and randomly chosen for each participant using an automatic MATLAB algorithm. After sleep, recall of the vocabulary was tested in a final retrieval phase using a cued recall procedure. For the details on EEG recording and pre-processing see Supplementary Methods. All of the following steps were accomplished with MATLAB (the MathWorks) using the open-source FieldTrip toolbox (Oostenveld et al., 2011).

Supplemental Methods:

EEG recording and preprocessing

EEG was recorded using a high-density 128-channel Geodesic Sensor Net (Electrical Geodesics, Eugene, OR). Impedances were kept below 50 kΩ. Voltage was sampled at 500 Hz and initially referenced to electrode Cz. Offline EEG preprocessing was realized using BrainVision Analyzer software. Data were re-referenced to an average-reference. The continuous EEG was epoched into intervals from 1,000ms before until 3,000ms after word onset. Trials affected by muscle or movement artifacts were manually removed. Eye blinks and movements of the pre-sleep EEG recordings were corrected using independent component analysis (Jung et al., 1998). For each phase (recall1, recall2 and TMR), segments were categorized based on the subjects' memory performance in the final retrieval phase into later remembered and non-remembered words..

Single-trial Phase Locking Value

The similarity of two signals was assessed as 1 minus the circular variance of difference in phase over time (Lachaux et al., 2000). The S-PLV is robust with regards to noisy data and allows for assessing similarity between two time windows in non-time-locked data (Michelmann et al., 2016). S-PLV ranges from 0 to 1, with 1 indicating perfect phase locking. Phase values were extracted using a complex Morlet wavelet of 6 cycles for frequencies between 1 and 20 Hz in steps of 0.5 Hz between 1000ms pre- to 3000ms after stimulus onset. For computational efficiency, the resulting phase values were down-sampled to 100 Hz. Lachaux and colleagues (2000) recommend assessing the S-PLV over 6–10 cycles of a given frequency to receive a good signal-to-noise ratio. At 5 Hz this would have resulted in a width of 1200 – 2000ms. Given that content-specific phase similarity during recall was observable in a rather narrow time window, we decided to use a 1 second sliding time-window (including 5 cycles). This should allow for obtaining an acceptable signal-to-noise ratio, while maintaining sufficient specificity (for the effects of varying the widths see Figure S3 (f + g)).

Slow Oscillations Detection

SOs were detected in all TMR data segments in EEG channel Fz. The signal was band-pass filtered between 0.5 and 4.0 Hz. The detection procedure resembled those earlier described in Bölsterli et al (2011) and Riedner et al., (2007). Half-waves were defined as negative deflections between two zero crossings. Corresponding quarter-waves were defined as the time between the negative peak and the following zero-crossing. Negative half-waves reaching at least an amplitude of −75μV were included in the analysis. Furthermore, only half-waves with a duration between 0.25 and 1 second (0.5–2 Hz) and which had a corresponding quarter-wave with a duration of more than 0.11 s (<2.25 Hz) were considered as SOs.

Source estimation

A spatial filter for each specified location (each grid point; $10mm^3$ grid) was computed based on the cross -spectral density, calculated for 5 Hz, using a complex Morlet wavelet, for all trials. As time windows of interest served the 1 second time-window derived from 'recall2' and the first second of TMR, given the most extended pattern of phase similarity effects during this period. Electrode locations for the 128-channel Geodesic Sensor Net EEG system were co-registered to the to the surface of a standard MRI template in MNI (Montreal Neurological Institute) space using the nasion and the left and right preauricular as fiducial landmarks. A standard leadfield was computed using the standard boundary element model (Oostenveld et al., 2003). The forward model was created using a common dipole grid ($10mm^3$ grid) of the grey matter volume (derived from the anatomical automatic labeling atlas (Tzourio-Mazoyer et al., 2002) in MNI space, warped onto standard MRI template, leading to 1457 virtual sensors. Data analysis was accomplished in the same way as before on sensor level.

TMR spectrum

As TMR during sleep seemed to have triggered reactivation processes in a recurrent fashion, we evaluated whether the similarity measures would fluctuate at a certain frequency .We performed a spectral analysis of the time-course of the phase similarity difference. We estimated the spectral power for frequencies between 0.25 and 16 Hz by multiplying a hanning taper to the Fourier transformation (-0.5 to 2.5 seconds) and evaluated potential peak frequencies, after correcting for the 1/f shape of the power spectrum. To correct for the 1/f shape of the power spectrum, this was accomplished on the first time-domain derivative of the data. Thereby power at every frequency bin is multiplied by its frequency, neutralizing the 1/f effect (Sleigh et al., 2001).

Cluster-based nonparametric permutation tests

This approach controls the Type I error rate with regard to multiple comparisons by clustering neighboring sensor pairs, exceeding a critical t-value in the same direction. For all included frequency bins (3-16 Hz), paired sampled t-tests were computed for any given electrode and for each time-point (-0.5 to 2.5 seconds). Thereby, clusters of contiguous sensors across participants were identified ($P < 0.05$, two-tailed). The cluster-level statistic was defined from the sum of the t values of the sensors in a given cluster. Only the cluster with the largest summed value was considered and tested against the permutation distribution (Monte-Carlo method, $P < 0.05$, two-tailed t-test).

Spectral power control analyses

As power differences can bias phase estimation, we tested whether there was a significant difference in power between conditions. We evaluated potential differences in oscillatory power for remembered versus non-remembered items, with regards to all steps of the experiment. Power values were extracted for 5 Hz using a complex Morlet wavelet of 6 cycles with regards to 2.5 seconds following stimulus onset. Oscillatory power for words remembered after 'recall2' was subtracted from power values of 'recall1' and contrasted against the difference of power-values of words not remembered during 'recall2' and their equivalent during 'recall1'. We specifically tested potential differences in oscillatory power for electrodes, which showed significant effects in phase-similarity between wakefulness and sleep (see Fig.3a; but please not that similar outcomes were obtained when adding the overall number of electrodes). We further estimated oscillatory power for the very same contrasts as utilized in the phase similarity analyses for recall1 vs. recall2 and recall2 vs. TMR respectively. With regards to the latter analysis oscillatory power for words remembered after sleep was subtracted from power values of 'recall2' and contrasted against the difference of power-values of words not remembered after sleep and their equivalent during 'recall2'.

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