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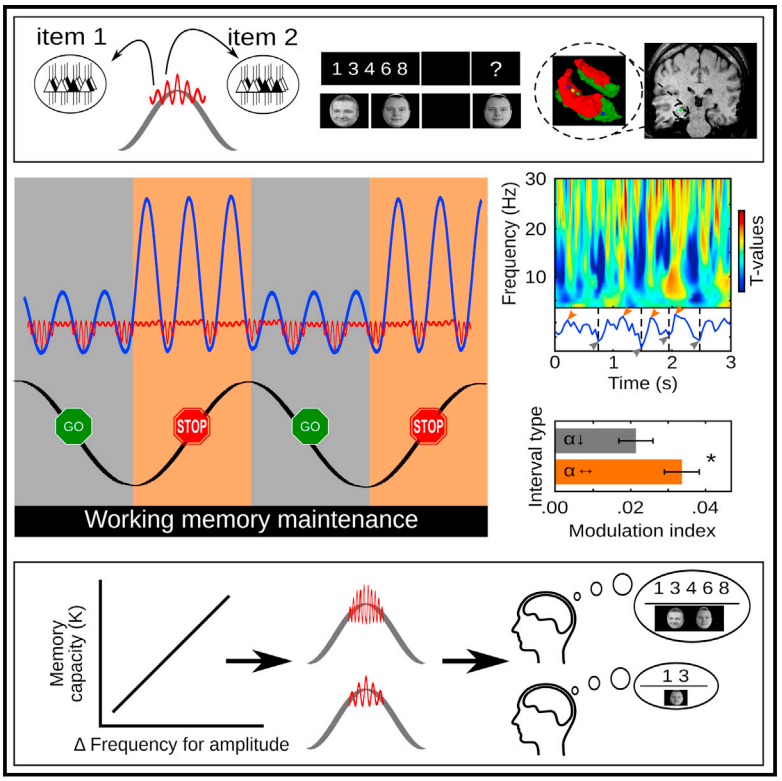
Rhythmic Working Memory Activation in the Human Hippocampus

Graphical Abstract



Highlights

- Working memory depends on rhythmic fluctuations in the human hippocampus
- Periods of memory activation are interleaved with periods of constant power levels
- Fluctuations between these two modes of processing are organized by delta rhythm
- A hierarchy of oscillations predicts successful performance and individual capacity

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In Brief

Leszczyński et al. find in two independent datasets that successful working memory maintenance is a dynamic process. It depends critically on rhythmic fluctuations between two different oscillatory modes of processing in the human hippocampus. These fluctuations predict successful task performance and individual working memory capacity.

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Rhythmic Working Memory Activation in the Human Hippocampus

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SUMMARY

Working memory (WM) maintenance is assumed to rely on a single sustained process throughout the entire maintenance period. This assumption, although fundamental, has never been tested. We used intracranial electroencephalography (EEG) recordings from the human hippocampus in two independent experiments to investigate the neural dynamics underlying WM maintenance. We observed periodic fluctuations between two different oscillatory regimes: Periods of "memory activation" were reflected by load-dependent alpha power reductions and lower levels of cross-frequency coupling (CFC). They occurred interleaved with periods characterized by load-independent high levels of alpha power and CFC. During memory activation periods, a relevant CFC parameter (load-dependent changes of the peak modulated frequency) correlated with individual WM capacity. Fluctuations between these two periods predicted successful performance and were locked to the phase of endogenous delta oscillations. These results show that hippocampal maintenance is a dynamic rather than constant process and depends critically on a hierarchy of oscillations.

INTRODUCTION

Working memory (WM) refers to the temporary retention of information that is no longer perceived. Therefore, successful performance in WM tasks requires keeping neural representations active during the maintenance interval. According to the multiplexing buffer model of WM, maintenance of multiple items relies on repeated interactions between neural oscillations in a high- and a low-frequency range (Lisman and Idiart, 1995; Lisman and Jensen, 2013). In detail, this model assumes that individual WM items are represented by cells firing synchronized in the gamma cycle (>30 Hz). Maintenance of item sequences depends on the sequential reactivation of these assemblies during consecutive phase ranges of simultaneous low-frequency oscillations.

The multiplexing buffer model assumes that representations of individual items (via high-frequency oscillations) do not occur during the entire cycle of low-frequency oscillations. Instead, these high-frequency oscillations are thought to be restricted to active "duty cycles," i.e., restricted phase ranges that correspond to higher levels of neural excitability (Mehta et al., 2002; Jensen et al., 2012, 2014). Between successive duty cycles, excitability is actively reduced in order to avoid overlap and interference between the representations of item sequences. Such an active inhibition has been related to oscillations in the alpha frequency range (8–12 Hz; Jensen et al., 2012; Sauseng et al., 2009; Thut et al., 2006; Jokisch and Jensen, 2007; Bonnefond and Jensen, 2012; Klimesch, 2012; Haegens et al., 2011; Spaak et al., 2012), although a similar function has been attributed to theta as well (Mehta et al., 2002). In particular, alpha power and phase reflect an attenuation of action potentials (Haegens et al., 2011) and high-frequency activity (Spaak et al., 2012). We will use the term "inhibition" when referring to this property of alpha oscillations. Notably, this term refers to an interpretation of alpha oscillations and not to electrophysiologically measured inhibition. A decrease of alpha power, in turn, reflects a release from inhibition (c.f. disinhibition). Furthermore, according to this alpha-inhibition model, increases in alpha power are accompanied by shorter duty cycles and decreases in alpha oscillations by longer duty cycles, respectively (Jensen et al., 2014; Figure 1A).

As a consequence of the separation of duty cycles by periods of inhibition, the amplitude of high-frequency activity is modulated by the phase of low-frequency activity, a phenomenon described as phase-amplitude cross-frequency coupling (CFC; Siegel et al., 2009; Axmacher et al., 2010). The multiplexing buffer model assumes a functional relevance of the CFC ratio, i.e., the frequency ratio between the most strongly modulated high-frequency activity (mHFA-max) and the most strongly modulating low-frequency oscillation (mLFA-max). This ratio (mHFA-max/mLFA-max) should be positively correlated with WM capacity, because longer low-frequency cycles allow representing longer sequences of items.

When alpha power is high, gamma-band activity occurs during a more restricted alpha phase range (shorter duty cycles), so that CFC strength is enhanced (Spaak et al., 2012; as described above, a similar function has previously been assigned to theta oscillations; Mehta et al., 2002). In the context of WM, this

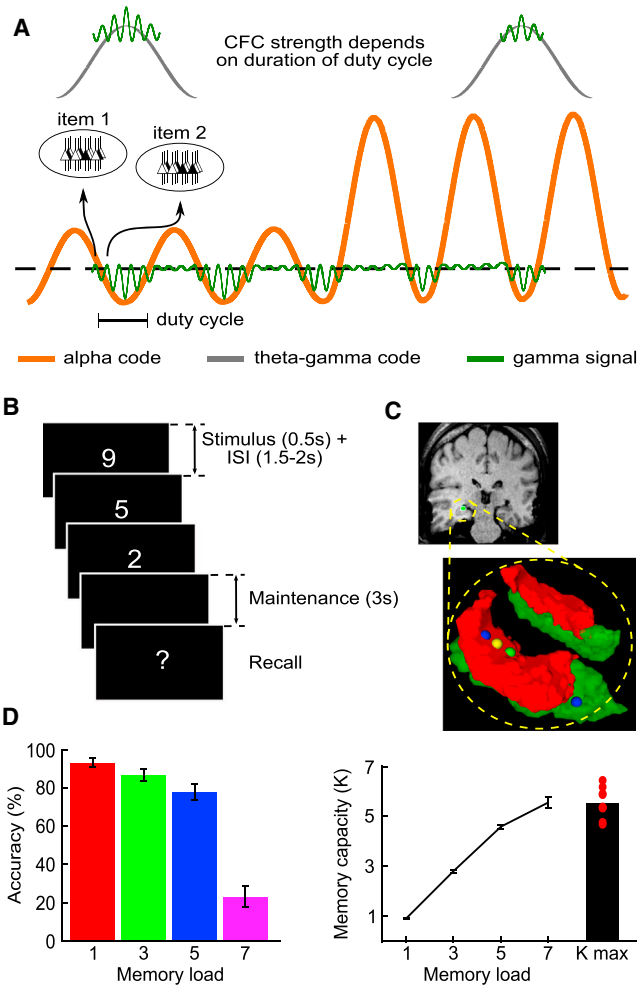

Figure 1. Theoretical Model, Experimental Paradigm, and Behavioral Results

(A) Theoretical model integrating the alpha inhibition and multiplexing buffer model of WM. The length of a duty cycle depends on power in the alpha frequency range. Low alpha power (on the left) results in longer duty cycles, whereas high alpha power (on the right) is associated with shorter duty cycle. The length of a duty cycle constrains gamma power, which in turn may be reflected as modulations to the theta-gamma code. In particular, long duty cycles may be observed as decreases in strength of the theta-gamma modulation index (modulating activity is broadly distributed across the low-frequency phase; upper left). In contrast, short duty cycles may be reflected by increases in CFC strength (upper right).

(B) Serial WM Sternberg task with consecutive presentation of one, three, five, or seven digits. Here, an example trial with three digits is presented. Participants needed to encode, maintain, and recall the sequence of digits.

(C) Coronal slice from one patient with hippocampal electrode contacts marked by spheres. A mask from the AAL atlas was used for identification of electrodes in the hippocampus (red; green mask, parahippocampal gyrus).

(D) Left: accuracy scores as a function of memory load. Right: WM capacity split across memory loads and the maximal value of capacity (k_{max}). Dots indicate single-subject scores. Error bars show SEM.

framework predicts that maintenance of longer sequences requires a reduction of alpha power, so that more items can be represented during longer duty cycles. Therefore, a high WM capacity should correspond to a decrease of CFC strength, because the modulated frequency would be more broadly distributed across the entire cycle of mLFA. WM capacity should be maximal if the duration of individual low-frequency cycles was as long as possible (lower frequencies of these oscillations), if high-frequency cycles were as short as possible (higher frequencies of these oscillations), or if CFC ratios were high. Thus, this model would lead to the prediction that WM capacity should be maximal when gamma-band activity is uniformly distributed across the entire cycle of low-frequency oscillations, i.e., in the absence of CFC. However, this may then lead to possible interference between consecutive representations of item sequences. This possible tradeoff between the demand to code as many items as possible over the entire phase range and the requirement to leave a temporal gap for non-coding phase ranges has not been investigated. Note that this model does not imply that during more extended duty cycles either CFC or the interacting oscillations of different frequencies become irrelevant, but it suggests that the phase-amplitude code is modulated by the length of duty cycles.

Here, we studied the dynamics of oscillatory duty cycles and their role for the maintenance of multiple items in WM. In two experiments, we presented participants with sequences of one, three, five, or seven digits (experiment 1), or one, two, or four faces (experiment 2). Items had to be maintained for 3 s and then recalled. WM capacity was measured as the amount of information that could be successfully maintained, controlled for guessing. During both experiments, we recorded intracranial electroencephalography (EEG) activity from the hippocampus of two independent groups of epilepsy patients, because previous results from neuroimaging (Nichols et al., 2006) and intracranial EEG (Axmacher et al., 2010; van Vugt et al., 2010) have shown that the hippocampus activates during WM maintenance of multiple items or item features. Furthermore, studies have shown that patients with hippocampal lesions have deficits when they are asked to maintain multiple items or associations between multiple item features in WM (e.g., Aggleton et al., 1992; Nichols et al., 2006; Olson et al., 2006). Thus, these studies suggest that the hippocampus is not only activated during WM tasks but also essential under certain conditions.

RESULTS

We will first report all results of experiment 1 (maintenance of digits; nine patients) and later describe the findings of experiment 2 (maintenance of faces; eight patients).

Load-Dependent Hippocampal Power Decrease: Memory Activation

We tested whether activity in the lower-frequency range (4–30 Hz) depends on WM load. We used surrogate-based cluster statistics to identify significant linear changes in power across memory loads (Maris and Oostenveld, 2007). Across our group of nine participants, we found four significant clusters ($p_{corrected} < 0.05$ each) in which power decreased linearly with increasing memory load (see Figures 2A, S1, and S3). These four clusters had an average duration of 208 ± 35 ms (mean ± SD across the four clusters),

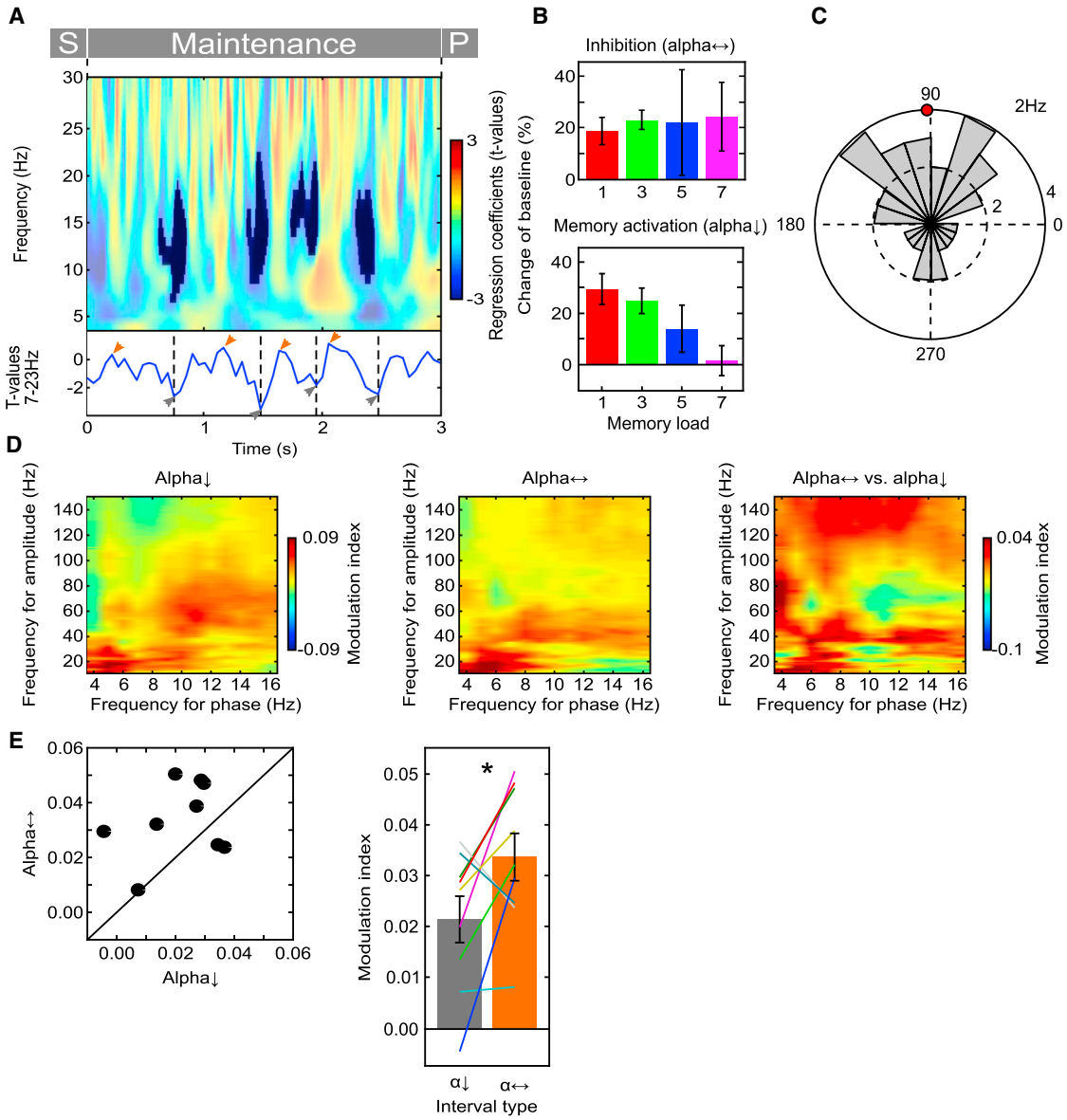

Figure 2. Difference between CFC during Periods with and without Load-Dependent Release from Alpha/Beta-Related Inhibition

(A) Load-dependent linear power decreases resulting from t statistic for regression coefficients calculated for each participant obtained by regressing the subject-specific spectro-temporal data on WM load. For each subject, data were averaged across trials of the same condition (loads 1, 3, 5, and 7). The subject-specific average across trials entered linear regression across memory loads. Note that the data were not averaged or summed across patients. In order to further test if the power fluctuations are consistent across the subjects, we calculated t statistic cluster-corrected for multiple comparisons. The color map reflects the results of this random-effects group-level t statistic (n = 9) showing that the power fluctuations calculated within each subject are consistent across the pool of patients. Bottom: time course of the linear regression averaged across frequencies where significant clusters were observed (i.e., between 7 and 23 Hz). See also Figure S1 for single subject data and Figure S3 for further analysis of the power fluctuations.

(B) Averaged alpha/beta power (7–23 Hz) during periods with (bottom) and without (top) load-dependent power decrease (memory activation and interleaving periods, respectively), showing that the difference between the two types of intervals is due to different distributions of power across memory loads. Error bars indicate SEM.

(C) Clustering of endogenous 2-Hz oscillation phases during periods with load-dependent memory activation (polar plot with 20 phase bins, each covering 18°). Data are pooled across nine patients and four clusters.

(D) Grand average CFC during time intervals with (left) and without (middle) load-dependent release from alpha/beta-related inhibition (memory activation periods). Color indicates CFC index (left, middle) and the difference between these periods (right), demonstrating higher CFC during periods without load-dependent disinhibition.

(E) CFC strength is lower during memory activation periods than interleaved periods. Left: each dot reflects data from a single subject with CFC calculated separately during time intervals with load-dependent power decrease (x axis) and without (y axis). Right: bar plot reflects group average of the CFC during intervals with (gray) and without (orange) load-dependent power decrease. Each line depicts a single subject. Error bars reflect SEM.

See also Figures S1 and S3.

covered frequencies in the alpha/beta range between 7 Hz and 23 Hz, and were distributed across the entire maintenance period with intervals of ~540 ms between them. Thus, the load-dependent decrease of alpha/beta power was organized into discrete short and repetitive time intervals rather than being sustained over the entire maintenance period.

These periods of linear power decrease were periodically interleaved with intervals without any indication of load-dependent power decrease (see Figures 2A and 2B). In addition, we observed a load-dependent increase of power in the gamma frequency range (30–150 Hz; see Supplemental Results). We suggest that the periods of load-dependent reductions of alpha-band activity reflect a rhythmic transient activation of working memory representations in the hippocampus. Below, we will report behavioral support for this interpretation.

Fluctuation of Memory Activation Periods at 2 Hz

The clusters showing load-dependent reductions of alpha/beta power occurred rhythmically every ~500 ms. Fast Fourier transform of the time course of the t scores for regression values indeed showed a peak in the power spectrum at 2 Hz, indicating dominant fluctuations at 2 Hz (see Figures S3B and S3C). We observed four clear cycles of load-dependent power reduction during the maintenance interval (Figure 2A, bottom). We tested whether the fluctuations of intervals showing release from alpha-dependent inhibition are consistently related to the phase of endogenous slow oscillations (Lakatos et al., 2005). We thus analyzed the phase angles of 2-Hz oscillations during these "memory activation" intervals using Rayleigh's test for non-uniformity of circular data. The phase angles were extracted during all time points when power effects occurred. The significant Rayleigh test shows that 2-Hz phases were indeed clustered during these intervals ($z = 3.19$, $p < 0.05$) and showed a preferred direction of 90° (Figures 2C, S3B, and S3C; Supplemental Results). This effect is consistent across the whole group of patients. Thus, intervals of memory activation occur during consistent phases of endogenous 2-Hz oscillations, indicating a hierarchy of hippocampal oscillations during WM processing.

Periodic Switching of Cross-Frequency Coupling Patterns

Next, we tested predictions from the multiplexing model of WM that posits interactions between the phase of low-frequency oscillations and the power of high-frequency activity as a mechanism for multi-item WM. Over the entire maintenance phase, we observed a significant increase of CFC as compared to surrogate data. The difference was found in a broad frequency range of 2–16 Hz for phase and 4–150 Hz for amplitude ($t(8) = 3.96$, $p_{corrected} < 0.05$), and also within a smaller range of 5–9 Hz for phase and 30–150 Hz for amplitude that was predicted by the original model of CFC (Lisman and Idiart, 1995; Lisman and Jensen, 2013; $t(8) = 3.99$, $p_{corrected} < 0.05$; Figure S3E).

Our initial observation that the load-dependent decrease of alpha/beta power (memory activation) occurs periodically in distinct temporal clusters led us to hypothesize that CFC might show a similar pattern of periodic fluctuations. To this end, we calculated CFC within 200-ms intervals centered on significant load-modulated alpha power reductions (memory activation) and interleaved intervals during which power was constant across memory loads. Indeed, we found that CFC strength was associated with alpha/beta load effects (Figures 2D and 2E). To quantify this effect, we directly compared CFC strength during periods with and without significant load-dependent decreases of alpha/beta power ($t(8) = 2.3699$, $p < 0.05$; Figure 2E). These results were not biased by differences in signal-to-noise ratio, as average load-independent power did not differ between the two interval types ($t(8) = 0.6126$, $p = 0.5571$).

To summarize, our data show that CFC strength is lower during periods in which alpha/beta-related inhibition is released with WM load. During these periods, CFC may support a process of memory activation during which modulated high-frequency activity (mHFA) is distributed across broader phase ranges of modulating low-frequency activity (mLFA), supporting maintenance of multiple items (see Figure 1A for the model, Figures 2D and 2E, and Discussion).

Correlations between CFC and WM Capacity

We calculated inter-individual correlations between individual WM capacity (Figure 1D, right) and CFC ratio, mLFA-max, and mHFA-max (Figure S5). Notably, we did not consider "raw" mLFA, mHFA and CFC frequency values but selected the frequencies at which CFC strength was maximally reduced during the memory activation periods as compared to the interleaved periods. These analyses were conducted separately for periods with and without load-dependent alpha/beta power effects (i.e., for memory activation periods and for the interleaved periods). The correlations were performed using Kendall's tau, which is a particularly conservative rank-correlation test to investigate correlations in small populations. It does not assume that the data are normally distributed (Abdi, 2007).

During memory activation periods, we observed a significant positive correlation between mHFA-max and WM capacity (Figure 3A; Kendall's tau = 0.59161, $p < 0.05$). No such relationship was observed for mHFA-max during periods without load-dependent release from inhibition (tau < 0.2, $p > 0.6$). Neither mLFA-max nor CFC ratio correlated with capacity during memory activation (all tau < 0.2, all $p > 0.5$) or during interleaved intervals (all tau < 0.6, all $p > 0.1$). In other words, patients with higher WM capacity showed modulations of gamma-band activity at higher frequencies during memory activation periods.

Replication Study: Periodic Switching Generalizes to WM of Novel Content

To test for the robustness of our results, we conducted the same analyses in a second independent dataset. Another group of eight different patients, again with hippocampal intracranial EEG (iEEG) electrodes, conducted a slightly different WM paradigm in which one, two, or four trial-unique unknown faces had to be maintained (Figure 4A). Results from this dataset have been published before (Axmacher et al., 2007, 2010).

We performed the same surrogate-based cluster statistics as in the first experiment to identify significant linear changes

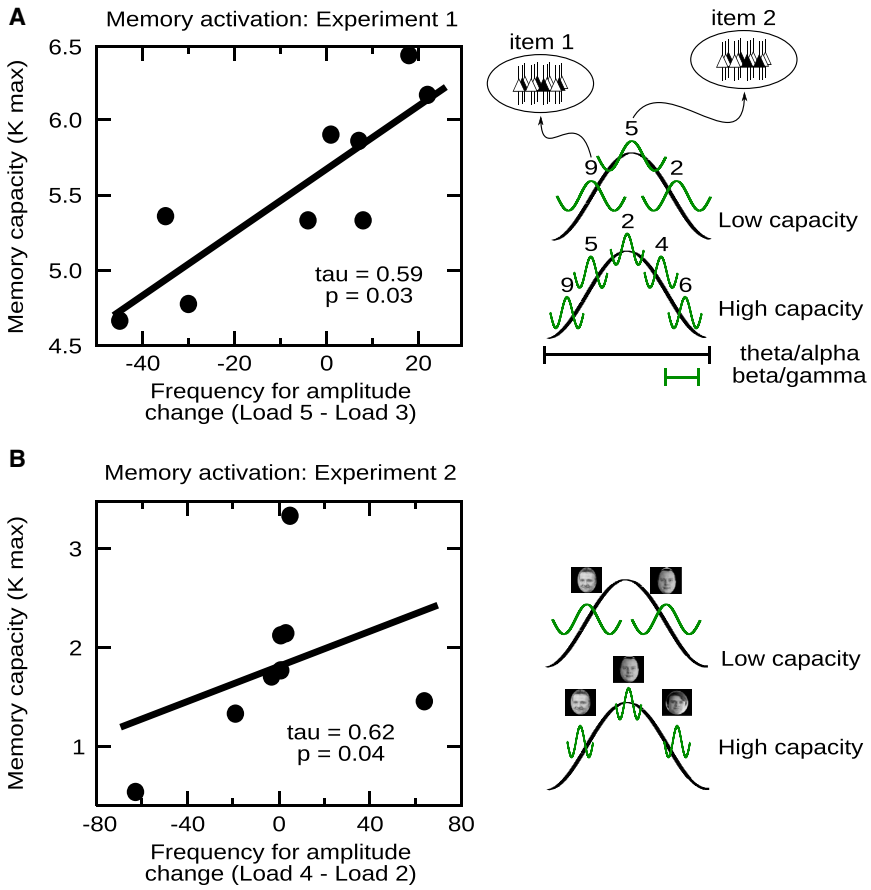

Figure 3. Behavioral Relevance of CFC

Experiment 1 (A) and experiment 2 (B). Left: during memory activation periods, individual WM capacity correlates with load-dependent changes in the frequency for amplitude. Right: this could be explained by faster cycles of high-frequency activity representing individual items in a sequence (top: digits; bottom: faces) and therefore increasing individual sequence WM span. See also Figure S5.

in low-frequency power across memory loads. We found three significant clusters ($p_{corrected} < 0.05$ each) in which power decreased linearly with increasing memory load (Figures 4D and 4E). These three clusters had an average duration of 627 ± 91 ms (mean ± SD across the three clusters), covered frequencies in the theta/alpha/beta range between 4 Hz and 20 Hz, and were distributed across the maintenance period with a center to center distance of 660 ms. Thus, similar to experiment 1, the load-dependent decrease of low-frequency power was organized into discrete short and repetitive time intervals rather than being sustained over the entire maintenance interval.

Next, we tested whether the periodicity of memory activation intervals was again related to the phase of endogenous slow-frequency oscillations. A fast Fourier transformation of the time series of regression t scores revealed a peak in the spectrum between 2 and 6 Hz (Figures S4B and S4C). We thus extracted the phases at this frequency range and analyzed whether phase values during memory activation periods were clustered. Indeed, a Rayleigh test showed phase values were not uniformly distributed ($z = 5.21$, $p < 0.005$) but were clustered at ~164° (Figure 4F). Memory activation intervals occurred during consistent phases of underlying slow oscillations. This replicates the finding of an oscillatory hierarchy during WM maintenance from our first experiment.

Next, we again compared the strength of CFC during memory activation and interleaved intervals. As in our first experiment, we observed that CFC was reduced during memory activation periods as compared to the interleaving intervals ($t(7) = 2.57$, $p < 0.05$). Again, we excluded that CFC was artificially driven by differences in (load-independent) power values (no difference in load-independent power across the two types of intervals: $t(7) = 0.79$, $p = 0.4551$).

Finally, we tested the behavioral relevance of CFC for WM capacity. We exactly reproduced our results from experiment 1. Again, during memory activation intervals, we observed a positive correlation between the frequency of mHFA-max and WM capacity (Kendall's tau = 0.62, $p < 0.05$; Figure 3B). Thus, higher WM capacities are related to higher frequencies of mHFA-max, which correspond to shorter cycles of amplitude-modulated high-frequency oscillations. Putatively, longer memory sets can be maintained when individual items are represented by faster mHFA cycles. We did not observe any correlation between WM capacity and mLFA frequency (tau = 0.38, $p = 0.26$) or CFC ratio (tau = 0.214, $p = 0.54$) in both types of interval.

Rhythmic Duty Cycles Are Critical for Successful WM Performance in the Hippocampus

To further assess the functional relevance of the observed periodicity of memory activation periods, we analyzed low-frequency power and CFC during incorrect trials (Figure 5). In these trials, we did not observe any load-dependent reduction of alpha/beta power. Interestingly, this was not due to an overall reduction of CFC, which was increased as compared to correct trials (0.033 versus 0.022: $t(14) = 2.07$, $p = 0.057$ in experiment 1 and 0.059 versus 0.034: $t(14) = 3.04$, $p < 0.01$ in experiment 2). This suggests that the temporally distributed reduction of CFC rather than the absolute CFC strength is critical for successful WM performance. Again, results were qualitatively identical in the two experiments (Figure 5).

DISCUSSION

Our data show that multi-item WM maintenance relies on a hierarchy of oscillations in the hippocampus reflecting switching between two separate functional processing modes. In two independent datasets, we found evidence for load-dependent linear

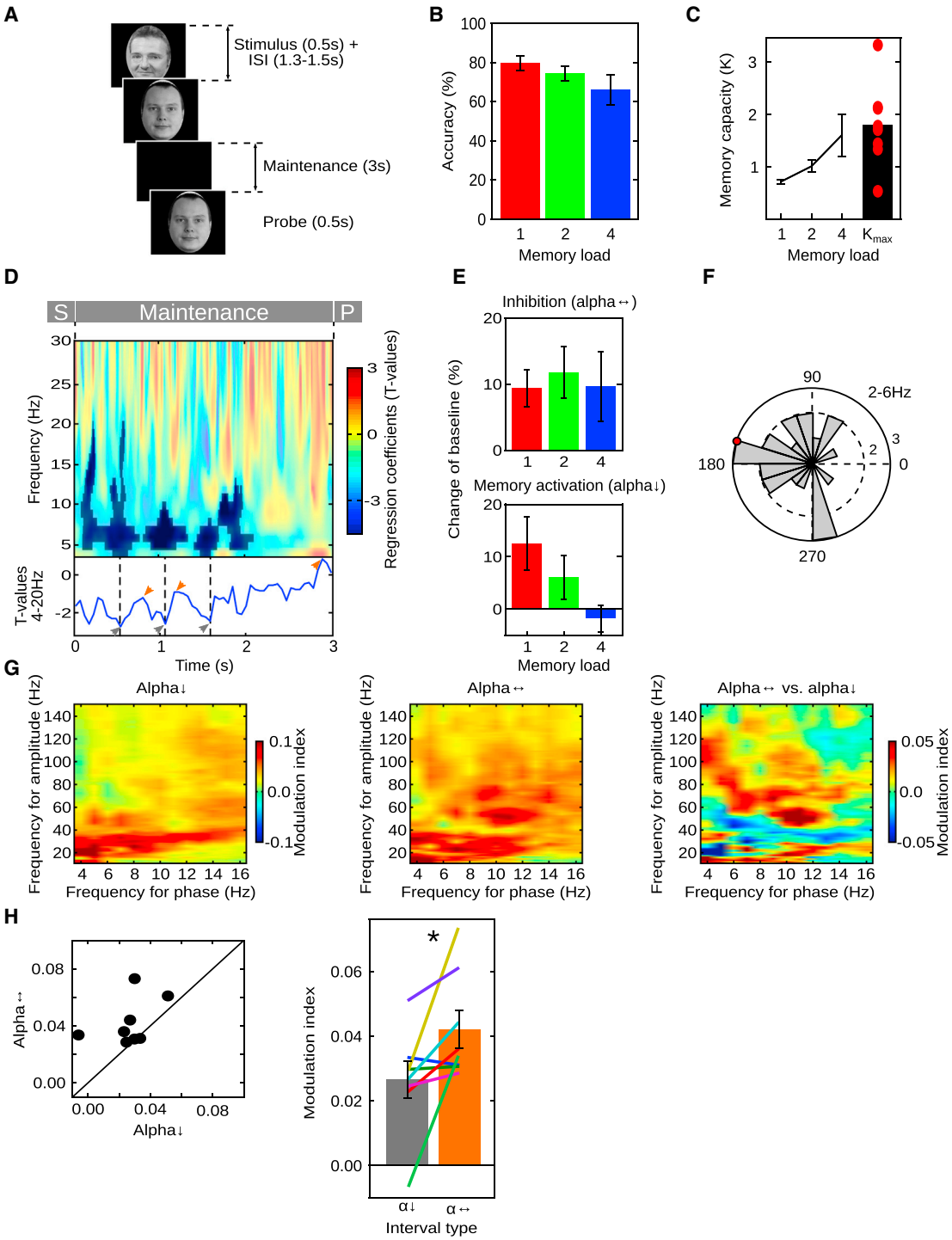

Figure 4. Replication Study

(A) Serial WM Sternberg task with one, two, or four unfamiliar faces as stimuli. Here, an example trial with two faces is presented. Participants needed to encode, maintain, and recall the stimuli.

(B) Accuracy scores as a function of memory load.

(C) WM capacity split across memory load and maximal value of capacity across load conditions (k_{max}). Dots indicate single-subject scores. Error bars show SEM.

(D) Periodic fluctuations between periods with and without load-dependent release from low-frequency-related inhibition. Top: regression coefficients indicating load effects on theta/alpha/beta power. Bottom: time course of averaged t values for regression coefficients between frequencies where clusters were observed

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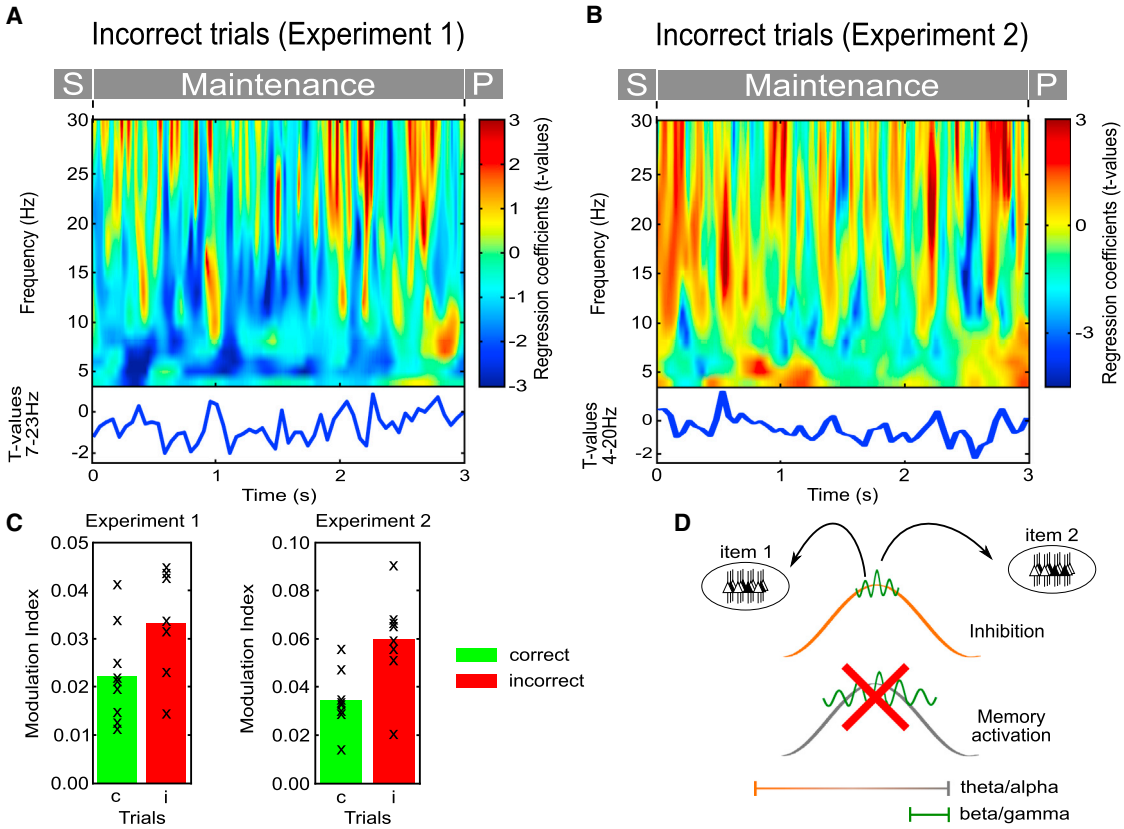

Figure 5. Data from Incorrect Trials: Absence of Memory Activation Periods during Incorrect Trials

(A and B) Top: regression coefficients indicating no linear decrease in power with memory load in experiment 1 and 2, respectively. Bottom: time course of averaged t values for regression coefficients between 7 and 23 Hz (A) and 4 and 20 Hz (B) in experiments 1 and 2, respectively. No significant differences were found.

(C) Bar plots indicate increased CFC during incorrect (red) as compared to correct trials during experiment 1 (left) and experiment 2 (right). Each "X" represents data from one single subject.

(D) Schematic depiction of incorrect trials with lack of memory activation periods. Absence of linear power decreases in the low-frequency range. Thus, there are no periods during which cycles of high-frequency activity are broadly distributed across larger phase ranges of low-frequency oscillations (corresponding to lower levels of CFC).

power decreases in the hippocampus that occurred periodically and phase-locked to endogenous delta oscillations. During these periods, the strength of cross-frequency coupling was overall reduced, while the load-dependent change in the peak modulated frequency was inter-individually correlated with WM capacity.

Alpha Inhibition Model

Our findings support a model in which alpha oscillations serve as a gating mechanism to prioritize relevant information (Jensen et al., 2012). We observed that during the maintenance interval, hippocampal low-frequency activity decreased with WM load (experiment 1: Figures 2A and 2B; experiment 2: Figures 4D

(4–20 Hz). Color arrows represent distinct periods, with gray depicting memory activation periods and orange reflecting interleaved intervals. See also Figure S2 for single-subject data. The horizontal gray bar represents the temporal structure of the paradigm (S, presentation of the last stimulus; P, probe for recall).

(E) Averaged alpha/beta/theta power (4–20 Hz) during periods with (bottom) and without (top) load-dependent power decrease (memory activation and interleaving periods, respectively), showing that the difference between the two types of intervals is due to different distributions of power across memory loads. Error bars indicate SEM.

(F) Clustering of endogenous 2- to 6-Hz oscillation phases during periods with load-dependent power decrease (memory activation). Data are pooled across eight patients and three clusters. See also Figure S3B.

(G) Grand average CFC during time intervals with (left) and without (middle) load-dependent release from theta/alpha/beta-related inhibition. Color indicates CFC index (left, middle) and difference between these periods (right), demonstrating higher CFC during periods without load-dependent disinhibition.

(H) CFC strength is lower during memory activation periods than interleaved periods. Left: each dot reflects data from a single subject with CFC calculated separately during time intervals with load-dependent power decrease (x axis) and without (y axis). Right: bar plot reflects group average of the CFC during intervals with (gray) and without (orange) load-dependent power decrease. Each line depicts data from one single subject. Error bars reflect SEM.
See also Figures S2 and S4.

and 4E). This decrease was not continuous across the entire maintenance phase but was organized into intervals that were equally spaced across the maintenance phase and interleaved with intervals showing no load-dependent disinhibition. Importantly, this power decrease and its periodicity were absent in incorrect trials (Figure 5). Several previous EEG and magnetoencephalography studies have provided consistent evidence that the power of parieto-occipital alpha oscillations increases when irrelevant information has to be suppressed in order to refine, or ''tune,'' representations of relevant items (Sauseng et al., 2009; Thut et al., 2006; Jokisch and Jensen, 2007; Bonnefond and Jensen, 2012; for reviews, see Jensen et al., 2012, 2014). These findings suggest that alpha oscillations reflect active inhibition, consistent with the negative correlations between alpha power and either action potential rates (Haegens et al., 2011) or high-frequency activity (Spaak et al., 2012). Conversely, release from alpha-related inhibition is required to process relevant information (Jensen et al., 2012). According to a slightly different perspective, reduced power of alpha oscillations improves information processing capacity via an increase of neuronal entropy (Hanslmayr et al., 2012). Beta oscillations have been reported to support similar inhibitory functions as alpha oscillations (Waldhauser et al., 2012). Our results show that alpha/beta activity was linearly reduced with WM load, suggesting that hippocampal alpha/beta activity plays a similar inhibitory role as alpha/beta activity in parieto-occipital networks. Furthermore, our results show that such a disinhibition is critical for successful performance in WM task.

We performed our analyses of low-frequency power signals within a broad frequency range (<30 Hz). Since there are no data available yet on alpha-related inhibition in the human hippocampus, we wanted to reduce prior assumptions regarding frequencies where the effects of functional inhibition might be observed. In particular, we suspected that both theta (Fell et al., 2011; Huang et al., 2014; Jensen et al., 2015; Mehta et al., 2002; van Kerkoerle et al., 2014) and beta (Bauer et al., 2014; Jensen et al., 2015; van Kerkoerle et al., 2014; Waldhauser et al., 2012) oscillations may show functional similar effects to alpha oscillations. Indeed, we found that the load-dependent reductions of power are not limited to the frequency range of 8–14 Hz but spread across a broader range of frequencies (7–23 Hz and 4–20 Hz in experiments 1 and 2, respectively). Furthermore, it should be noted that there are no data regarding frequencies of oscillations in the hippocampus that correspond to the alpha range in the neocortex. The current results suggest that the hippocampus exhibits functionally similar oscillations across a broader frequency range.

Multiplexing Buffer Model

CFC was reduced during the memory activation periods as compared to the interleaving periods (Figures 2D, 2E, 4G, and 4H). This result may appear surprising because CFC has been related to active maintenance of relevant information by the multiplexing buffer model (Lisman and Idiart, 1995; Lisman and Jensen, 2013). However, weaker CFC reflects broader distributions of mHFA across the phase of mLFA, maximizing the number of items that can be simultaneously maintained (Figure 1A). Thus, our finding of weaker CFC during intervals with load-dependent hippocampal disinhibition is consistent with an increasing involvement of the hippocampus for representing longer sequences (see also the Supplemental Discussion).

Relation of CFC to Working Memory Capacity

The current results confirm one very prominent prediction from the multiplexing model of WM. In two independent datasets, we observed inter-individual correlations between WM capacity and a central measure of CFC, the peak frequency for modulated activity. In particular, we observed that higher numbers of items that participants were able to maintain (with increasing load) were associated with more pronounced increases of the frequency for amplitude. Patients who show stronger increases in the peak frequency-for-amplitude from lower to higher loads are capable of maintaining longer sequences in working memory. Importantly, this relation was observed for different types of material: We observed similar correlations during maintenance of simple and highly familiar visual features (digits) and for trial unique, novel, and complex stimuli (faces). Given that the frequency-for-phase was constant across memory loads, the increasing peak frequency-for-amplitude for participants with higher WM capacity may be associated with more distinct representations at increasing memory load. A very interesting follow-up question is whether this mechanism is specific to the hippocampus or whether it generalizes to other brain networks that are involved in visual working memory such as prefrontal, parietal, or occipital cortices.

Periodic Switching between Two Modes of Processing in the Human Hippocampus

The current finding of slow fluctuations between two different oscillatory regimes phase-locked to endogenous delta oscillations might reflect a similar process of switching between different modes as observed in rodents in the firing pattern of entorhinal grid cells (De Almeida et al., 2012) and CA1 local field potentials (Colgin et al., 2009). Entorhinal grid cells have been found to operate in two modes: (1) a predictive mode in which grid cells represent a location ahead of an animal and (2) a short-term memory mode in which the cells represent a position just passed. Importantly, both modes are organized by theta and gamma oscillations (De Almeida et al., 2012). Since theta oscillations have been suggested to correspond to the human delta rhythm (Lega et al., 2012; Watrous et al., 2013), the current results might reflect a homologous mechanism of periodic switching between two modes in the human hippocampus. Colgin et al. (2009) observed that gamma oscillations in the CA1 also alternate between two modes. Fast gamma (~65–140 Hz) oscillations in CA1 were synchronized with fast gamma in medial entorhinal cortex (MEC), whereas slow gamma oscillations in CA1 (~25–50 Hz) were synchronized with slow gamma in CA3. Importantly, these two types of gamma were locked to distinct phases of the CA1 theta rhythm. These results suggest that information routing between CA1 and CA3/MEC fluctuates periodically with theta phase, providing a mechanism for temporal segregation of information from different sources. The current CFC attenuation observed during memory activation intervals coherently locked to delta phase might reflect an analogous mechanism for temporal segregation of potentially interfering

information in the human hippocampus (e.g., already maintained information and incoming new information).

Periodic Gating by a Hierarchy of Oscillations in the Hippocampus

The hippocampus shows a periodic switching between power decrease periods supporting maintenance of relevant information and periods during which constant power is maintained regardless of memory load. This rhythmic switching was locked to the phase of endogenous delta oscillations (Figures 2C, 4F, S3B, S3C, S4B, and S4C; Supplemental Discussion), corresponding to an oscillatory hierarchy (Lakatos et al., 2005) in which the phase of delta oscillations is coupled to the strength of CFC between two other oscillations.

Slow-frequency oscillations have been suggested to serve as a gating mechanism during WM (Raghavachari et al., 2001, 2006). Here, we report that the hippocampus is being gated by a hierarchy of oscillations fluctuating with delta rhythm rather than by continuous and sustained oscillations. What might, under natural conditions, be the benefit of the rhythmic fluctuation of load-dependent disinhibition; i.e., why is memory activation not sustained across the entire maintenance period? Of course, we can only speculate about possible functional advantages of this periodicity. It seems likely that the memory activation interferes with some other process, like an updating of memory representations that might depend on interactions between the hippocampus and prefrontal regions. Maintenance of multiple items during periods when the hippocampus is released from inhibition may benefit from a general strengthening of WM representations. The very same representations might then be updated for potentially new, upcoming information during periods with high levels of hippocampal inhibition across all memory loads. Although somewhat speculative, this interpretation has a precedent in other domains of cognition: A similar mechanism of periodic switching between strengthening and inhibiting of neural responses has been found to maximize limited sensory capacities during visual perception (Busch and VanRullen, 2010).

The current study finds evidence for a periodic switching between functional modes in two independent datasets recorded from the human hippocampus. Note that our conclusions regarding rhythmic switching during WM maintenance are limited to the human hippocampus. In particular, our data by no means exclude that there may be another brain area outside of the hippocampus (e.g., prefrontal or occipital cortex) that exhibits continuous rather than rhythmic activity patterns during WM maintenance. This continuous WM buffer may then drive the hippocampus during one of the hippocampal states (e.g., the memory activation state), but not the other.

Taken together, our results show periodic fluctuations between two different oscillatory regimes in the human hippocampus during WM maintenance, which are critical for successful performance. During memory activation periods, we observed decreased CFC strength and interindividual correlations between CFC parameters and WM capacity. In contrast, the interleaved periods were characterized by high levels of hippocampal inhibition and increased CFC strength. The human hippocampus supports multi-item WM by periodically switching between these two functions at delta frequency.

EXPERIMENTAL PROCEDURES

Experiment 1

Details about participants, recordings, and analyses can be found in the Supplemental Experimental Procedures.

Task

Participants performed a modified Sternberg WM task with digits serving as stimuli. Each trial contained a sequence of either one (load 1), three (load 3), five (load 5), or seven (load 7) digits. Each item was presented for 0.5 s, followed by an inter-stimulus interval of a duration that was randomly jittered between 1.5 s and 2 s. Once the sequence had been presented, participants had to maintain the representation of the entire memory set for the duration of a retention interval lasting 3 s. Next, a question mark informed participants to type all digits in correct order using a keyboard (Figure 1B). We tested free recall of entire sequences because the hippocampus is known to be particularly relevant for sequence memory (Eichenbaum, 2000; Kumaran and Maguire, 2006). A total of 120 trials were presented (30 trials per load condition) in random order. During the experiment, iEEG signals from depth electrodes (Figure 1C) as well as from the linked mastoids were continuously recorded. The study was approved by the local medical ethics committee, and all patients gave written informed consent.

Time-Frequency Analysis

Continuous EEG data were segmented into 7-s-long epochs with a 2-s prestimulus period and a 5-s poststimulus interval relative to presentation onset of the last item in each trial. Such relatively long segments were used to minimize edge effects. The epoched data were convolved with four-cycle Morlet wavelets from 4 Hz to 150 Hz in steps of 1 Hz. Power values were normalized with respect to prestimulus time windows from −1.3 s to −0.3 s separately for each patient, frequency, and trial. Since we were interested in the activity specific to the retention interval, activity during the time period after the offset of the last item in each trial and across the entire 3-s maintenance period was analyzed. Based on our a priori hypothesis of linear decreases of alpha/beta activity with memory load, we calculated regression coefficients across the four memory loads. To this end, condition-specific data for each participant were averaged across trials. We then calculated regression coefficients within each participant, regressing subject specific spectro-temporal data on working memory load. In order to test reliability of the subject-specific linear decreases of power across memory loads, we further calculated non-parametric surrogate statistics with cluster correction for multiple comparisons (Maris and Oostenveld, 2007; Supplemental Experimental Procedures).

Cross-Frequency Coupling

In order to investigate if WM maintenance of sequence information depends on the coupling of high-frequency power to low-frequency phase, we calculated a modulation measure according to a previously described procedure and compared these results to surrogate data obtained by randomly re-assigning trials for amplitude and trials for phase (Axmacher et al., 2010; Supplemental Experimental Procedures).

As load effects on power in the alpha/beta frequency range appeared to be periodically modulated (Results; Figures 2A, 2B, 4D, and 4E), we were interested if the CFC modulation index shows similar periodic fluctuations. Thus, we computed the CFC calculated within 200-ms windows centered on memory activation periods and interleaved intervals. Concatenated results were compared between two types of interval using pairwise t tests within the frequency-frequency range predicted by the multiplexing model (Lisman and Idiart, 1995; Lisman and Jensen, 2013).

The multiplexing buffer model predicts that participants with a high WM capacity can maintain more items because more cycles of high-frequency activity "fit" onto one cycle of low-frequency activity for these participants. In other words, this model predicts that participants with high WM capacity are characterized by lower peak modulating frequencies, while the peak modulated frequencies remain constant. Alternatively, they could show the same mLFA frequency as participants with a lower capacity but higher mHFA frequencies. To test these predictions, we quantified for each participant the frequencies of

mLFA-max and mHFA-max at which the difference of CFC was maximal between periods showing load-dependent disinhibition and interleaving periods, and we extracted the CFC ratio at this point. Then, we calculated Kendall's tau to quantify the relationship between individual WM capacity (as described above) and CFC ratio, mHFA-max, and mLFA-max.

Experiment 2

We aimed to replicate the observed effects in an independent dataset. A new group of patients (n = 8, mean age of 43 years, two females) implanted with electrodes in the hippocampus performed a similar WM task (Figure 4A; for details, see Axmacher et al., 2007, 2010). All patients had well-defined lateralized ictal onset zones (four patients had a seizure-onset zone within the left hippocampus and the other four in the right hippocampus). There were three main differences regarding the previous design: (1) Instead of simple and highly familiar stimuli used in experiment 1, we now used images of faces that were complex materials consisting of multiple features and unfamiliar to our participants; (2) participants were probed with a stimulus and were supposed to indicate if the probed face was presented in the WM set or if the face was new (a two-alternative forced choice task [2AFC] instead of a whole report task); and (3) memory load was one, two, or four faces. Timing of stimulus presentation and retention duration were the same as in experiment 1. All preprocessing steps and statistical analyses were the same.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Results, Supplemental Discussion, Supplemental Experimental Procedures, and five figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.09.081.

AUTHOR CONTRIBUTIONS

M.L., J.F., and N. A. designed the study, analyzed the data, and wrote the manuscript.

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