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Brain state-dependent recruitment of high-frequency oscillations in the human hippocampus

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Abstract

Ripples are high-frequency bouts of coordinated hippocampal activity believed to be crucial for information transfer and memory formation. We used intracortical macroelectrodes to record neural activity in the human hippocampus of awake subjects undergoing surgical treatment for refractory epilepsy and distinguished two populations of ripple episodes based on their frequency spectrum. The phase-coupling of one population, slow ripples (90-110 Hz), to cortical delta oscillations was differentially modulated by cognitive task; whereas the second population, fast ripples (130-170 Hz), was not seemingly correlated to local neural activity. Furthermore, as cognitive tasks changed, the ongoing coordination of neural activity associated to slow ripples progressively augmented along the parahippocampal axis. Thus, during resting states, slow ripples were coordinated in restricted hippocampal territories; whereas during active states, such as attentionally-demanding tasks, high frequency activity emerged across the hippocampus and parahippocampal cortex, that was synchronized with slow ripples, consistent with ripples supporting information transfer and coupling anatomically distant regions. Hence, our results provide further evidence of neural diversity in hippocampal high-frequency oscillations and their association to cognitive processing in humans.

Introduction

Ripples are high-frequency (100-200 Hz) network oscillations in the hippocampus and parahippocampus of mammals that take place during quiet wakefulness and slow-wave sleep (O'Keefe and Nadel, 1978, Buzsaki and Silva, 2012), and provide short-lived windows of synchronous activation for large neuronal populations, ideally suited for transferring information and inducing plasticity (Buzsaki, 1989). Ripple episodes have been linked to learning and memory consolidation, in particular during sleep (Axmacher et al., 2008, Girardeau et al., 2009). However, recent evidence also supports their active role in planning (Dragoi and Tonegawa, 2010) and decision-making (Jadhav et al., 2012, Singer et al., 2013) in animal models.

These high-frequency episodes were initially described in rodent models in vivo (O'Keefe and Nadel, 1978, Buzsaki et al., 1983), and later on the cellular mechanisms were described in vitro (Draguhn et al., 1998, Maier et al., 2003, Maier et al., 2011). Until recently, human studies were uncommon because of the deep anatomical location of the hippocampus and the fact that its electrical potentials cannot be detected with scalp recordings. However, nowadays it is sometimes possible to study neural activity from the human hippocampus in refractory epilepsy patients with intracranial electrodes implanted for clinical diagnostics. This technical breakthrough has confirmed the existence of ripples in the human hippocampus, with similar properties to those described in animal models (Bragin et al., 1999, Le Van Quyen et al., 2010, Kucewicz et al., 2014). Indeed, cortical recordings have reported ripple oscillations, not only in the hippocampus, but also in the neighboring rhinal cortex (Staba et al., 2002, Axmacher et al., 2008, Kucewicz et al., 2014). Importantly, human recordings have been conducted during memory consolidation experiments; thus establishing a direct link with behavior (Axmacher et al., 2008, Kucewicz et al., 2014).

Ripples are particularly abundant during deep sleep and quiet wakefulness (O'Keefe and Nadel, 1978). However, recent evidence from animal studies has shown that ripples are

causally important for memory consolidation during both sleep (Girardeau et al., 2009) and wakefulness (Jadhav et al., 2012). Additionally, a recent study has shown that hippocampal high-frequency oscillations correlate with cognitive processing in human recognition memory (Kucewicz et al., 2014). Hence, we tested the hypothesis that ripples are modulated during wakefulness by various cognitive tasks that likely involve different brain states. Accordingly, we recorded hippocampal neural activity from epileptic patients implanted with intracortical macroelectrodes for surgical treatment. We found two populations of ripples, of which only one, slow ripples, was seemingly modulated by cognitive task. Additionally, we show that oscillatory activity induced by ripple oscillations propagates anatomically in relation to cognitive task. Indeed, during quiet rest ripples were not associated with significant oscillatory activity in neighboring hippocampal areas; whereas during memory recall ripples were accompanied with significant oscillatory activity in neighboring hippocampal areas; and during directed attention, concomitant activity in high frequency bands could be detected in parahippocampal areas. Hence, our results show diversity of hippocampal high-frequency episodes and suggest a distinct role for ripples in differentially recruiting cortical regions according to brain state.

1. Methods

1.1. Participants. Intracranial recordings were obtained from six neurosurgical patients with intractable epilepsy (Table 1) at the Epilepsy Department of the Grenoble Neurological Hospital (Grenoble, France). All patients were stereotactically implanted with multi-lead iEEG depth electrodes. All electrode data exhibiting pathological waveforms were discarded from the present study. We followed our routine procedures to ensure that analysis in this study was performed using recordings that originated from healthy cortical tissue and that the interpretations of data are not biased by pathological traces related to epileptic activity. This was achieved by using several procedures. We first used automatic identification of electrodes that contained epileptic spikes based on straight-forward amplitude thresholds (typically >10 S.D. on the raw voltage signal). In addition, we visually scanned all the data obtained in each subject in search for artefactual and pathological traces, assisted by expertise from neurologists (Dr. P. Kahane and medical team). Furthermore, after surgery was performed in order to remove the identified focus of the seizure, we double checked that none of the sites kept for our analysis were part of the resected area. For analysis, we removed all periods where any suspected epileptic activity in either the hippocampal electrode or epileptic region was detected. Hence, for each condition we eliminated about 15 seconds of recording, which represents less than 5% of the total recording time per condition (300 sec). We did not find any difference in epileptic discharges between conditions in either total time ($p = 0.5$) or proportion of time ($p = 0.2$, Friedman test). All participants provided written informed consent and the experimental procedures were conducted in accordance to the Institutional Review Board and the National French Science Ethical Committee (CPPRB). All experimental protocols were approved by the CPPRB.

1.2. Stimuli and Experimental Design. We explored the modulation of ripples during different cognitive tasks: (i) quiet rest, subjects were instructed to rest quietly but awake with their eyes closed during 5 min; (ii) autobiographic memory recall, subjects were instructed to remember for 5 min all events experienced during the day in order and detail, sitting quietly with their eyes closed. After this period, participants were asked to describe the events experienced to

allow the experimenter to determine the clarity of the memory; (iii) directed attention, subjects were instructed to find a target letter embedded in an array of distracter letters as fast as possible, the target being a gray letter 'T', presented in all trials. Stimuli and paradigm were adapted from a previous study (Treisman and Gelade, 1980). Briefly, two conditions were presented: an easy search and a difficult search. In the easy condition, the target was gray and all distracters were black. In the difficult condition, both target and distracters were gray. The difficult and easy condition stimuli were presented randomly for a fixed duration of 3 s and interstimulus interval of 1 s. To dissociate correct from wrong responses the subjects were required to indicate whether the target was located in the upper or lower half of the display by pressing one of two response buttons. Stimuli were displayed on a 19 inch computer screen located 60 cm away from the subject. Each condition consisted of a 5 min recording block (Ossandon et al., 2011). Analysis of the behavioral data showed that correct target detections (achieved within the fixed 3 s time window) dropped from 92% hits in the easy search to 84% hits in the difficult search. For analysis, we considered data from the whole recording period during the task, including the inter-stimulus intervals. Thus, we did not measure any behavioral parameter in the quiet rest or memory recall tasks. Right after the quiet rest task, subjects were asked if they felt reinvigorated and relaxed. Similarly, after the autobiographic memory recall, participants were asked to describe in detail the events recollected to allow the experimenter to determine memory precision. Only in the directed attention task we quantitatively measured performance. However, we did not attempt to correlate it with high frequency activity in the hippocampus.

1.3. Electrode implantation. Eleven to 15 semi-rigid multi-lead electrodes were stereotactically implanted in each patient. The stereotactic-EEG (SEEG) electrodes were 0.8 mm in diameter and, depending on the target structure, consisted of 10-15 contact leads, 2-mm wide, and 1.5-mm apart (DIXI Medical; Besançon, France). Electrodes were anatomically localized by aligning the pre-operative with the post-operative (i.e., electrodes in place) structural MRIs of each patient and their localization is provided in MNI coordinates (Fonov et al., 2011).

1.4. SEEG recordings. Intracerebral recordings were conducted using a video-SEEG monitoring system (Micromed; Treviso, Italy), which allowed the simultaneous data recording from 128 depth-EEG electrode sites. The data were band-pass-filtered online (0.1-200 Hz) and sampled at 512 Hz in 6 patients. At the time of acquisition the data was recorded using a reference electrode located in white matter, each electrode trace was re-referenced with respect to its closest neighbor (i.e., bipolar derivation). The spatial resolution achieved by the bipolar SEEG is of the order of 3 mm (Jerbi et al., 2009).

1.5. Electrode selection. Hippocampal electrodes were selected for analysis according to the following criteria. First, the electrode localization normalized to the MNI space was located on the hippocampal region according to a standard atlas (<http://www.alivelearn.net/xjview>). Then, electrode locations were verified by visual inspection in neuroimages from every subject. Finally, the median amplitude of oscillatory events detected in those electrodes (see below) was larger than 3 S.D. of events detected in all electrodes from the same subject (Figure 1). Importantly, recordings sites from the same recording electrode (intraelectrode) or from different recording electrodes (interelectrode) could be located within the hippocampus. For cross-correlograms and ripple-induced spectrograms we used as reference the recording site in which ripple events exhibited the largest amplitude in every subject. In all cases we used

bipolar derivation to generate an analytical signal for further processing, avoiding the pooling of recording sites with common electrical references. For example, for the consecutive recording sites: h1, h2, h3, and h4, we only considered for analysis the bipolar signal from h1-h2 and h3-h4, and not h2-h3 (Table 2).

1.6. Extraction of ripple episodes. Procedures for the detection of ripples have been extensively described in previous studies in rats, monkeys, and humans (O’Keefe and Nadel, 1978, Staba et al., 2002, Axmacher et al., 2008, Buzsaki and Silva, 2012, Logothetis et al., 2012). We used two methods based on the previously described ones, with some modifications (Supplementary Figure 1). In the first method, the signal recorded from the hippocampus was filtered between 80-180 Hz, according to the detection of ripples in humans and monkeys (Axmacher et al., 2008), using a zero phase-shift, non-causal finite impulse filter response with 0.5 Hz roll-off. The envelope of the signal was obtained by rectification (i.e., the signal was centered and its absolute value was computed) and low-pass filtering at 20 Hz with a 4th-order Butterworth filter. This procedure yields a smooth envelope of the filtered signal, which was then z-score normalized using the mean and standard deviation of the whole signal, for each electrode and condition. Epochs during which the normalized signal exceeded a 3.5 S.D. threshold during more than 20 ms were considered oscillatory events (i.e.; ripples). Additionally, we used a second method for ripples detection, since the use of broadband intervals to detect oscillatory events is biased towards low frequencies, due to the $1/f^n$ drop-off in frequency power. Specifically, before applying the first method, we normalized the amplitude of the spectral distribution. For this, we extracted the amplitude of the recorded signal in the Fourier space and normalized it by using the smoothed amplitude with a 10 Hz-span, and then reconstructed the original signal. We used *equation 1*:

$$WS(t) = iFFT \left(\frac{FFT(S(t))}{SMA(FFT(S(t)))} \right)$$

where $WS(t)$ is the ‘whitened’ signal, FFT is the fast Fourier transform, $iFFT$ is the inverse fast Fourier transform, $S(t)$ is the original signal, and SMA is the simple moving average of the module; this last one was computed in *equation 2* as:

$$SMA(f) = \frac{1}{2n} \sum_{i=-n}^n |F(f+i)|$$

where $F(f)$ is the fast Fourier transform of the original signal. The parameter n was fixed to 5 Hz, to get a 10 Hz smoothing span. In this way, we obtained a signal with the original phase and ‘whitened’ amplitude. We then applied on the resulting signal the first method above to detect oscillatory events (Supplementary Table 1). Note that the normalized signal was used only for the detection of ripple events. For all subsequent analysis of detected events, we processed the non-normalized, raw signal.

1.7. Ripples clustering and time-frequency analysis. The two detection methods might extract different populations of events. Hence, we carried out cluster analyses to evaluate their performance. For this, all events detected were pooled, excluding repeated events; that is, those detected within a refractory time window (50 ms). In order to precisely determine the mean frequency, amplitude, and duration of each oscillatory event independently of the detection method, we performed an independent spectral analysis using complex Morlet-wavelets. The rationale of that was to prevent biasing the calculation of ripple properties in

the cluster analysis (i.e., using the normalized or non-normalized amplitude of events). This method is suitable to accurately describe time-frequency properties since it maintains constant the number of cycles for each analyzed frequency range, thus avoiding biasing results by the period duration. We used 7 cycles as it is the most commonly used parameter to detect high frequency activity (> 40 Hz). Thus, by using the mean power at each frequency band per event, we separated the events into clusters. To this end, we used both a hierarchical dendrogram, with weighted average Euclidean distances, and a K-means algorithm (Figure 1). Results from these analyses were consistent, per electrode and condition, yielding two distinct clusters with maximum frequencies centered at 100 and 150 Hz. We repeated this method for each electrode and condition to obtain ripple densities. To evaluate the frequency spectrum of the whole signal per condition and electrode, we used a multitaper method [4 s-window, 0.5 Hz smooth, 3 tapers (Mitra and Pesaran, 1999)]. This method has good spectral concentration and yields smooth results, with good bias-variance characteristics (Bokil et al., 2007).

1.8. Nested-Frequency Analysis. To assess cross-frequency phase-amplitude coupling (Figure 2), we calculated a modulation index (MI) as previously described (Tort et al., 2008, He et al., 2010). This method has been used recently, for example, in a study of human hippocampal oscillatory activity (Staresina et al., 2015). For each frequency pair f_P and f_A we filtered and Hilbert-transformed a time series $x(t)$ in the corresponding frequency bins $|f_P|$ and $|f_A|$. Instantaneous phase ($\varphi_{f_P}(t)$) and instantaneous amplitude ($A_{f_A}(t)$) time series were obtained from the corresponding Hilbert-transform. Sample-by-sample values of $\varphi_{f_P}(t)$ in a 200 ms window around every ripple peak were drawn and binned into twenty 0.1π -width intervals from $-\pi$ to π , and the concurrent $A_{f_A}(t)$ values were averaged within each phase bin. We denote as $\langle A_{f_A} \rangle_{\varphi_{f_P}(j)}$ the mean $A_{f_A}(t)$ value at phase bin $\varphi_{f_P}(j)$ ($j = 1, 2, \dots, 20$). The modulation index (MI) is a measure which describes the deviation of $\langle A_{f_A} \rangle_{\varphi_{f_P}(j)}$ from a uniform distribution in equation 3:

$$MI = \frac{H_{\max} - H}{H_{\max}}.$$

H , the entropy of $\langle A_{f_A} \rangle_{\varphi_{f_P}(j)}$ distribution, is defined by $H = -\sum_{j=1}^N p_j \log p_j$, where $N = 20$ (i.e., the number of phase bins), and p_j is given by equation 4:

$$p_j = \frac{\langle A_{f_A} \rangle_{\varphi_{f_P}(j)}}{\sum_{k=1}^N \langle A_{f_A} \rangle_{\varphi_{f_P}(k)}}.$$

H_{\max} is the maximum possible entropy, or $H_{\max} = \log N$. In order to control for background phase-amplitude coupling, we carried out the same procedure permuting randomly the inter-event interval (1000 permutations). For each electrode and condition we subtracted the mean background modulation. Finally, we tested if there was a consistent tendency in modulation of the MI across conditions. For this, we computed for each phase–amplitude pair the trend Page test across electrodes and conditions (tested if there exists the following tendency among conditions: $da > amr > qr$, see next section). Thus, for each pair we obtained rho and L values (see equation 5), which we further corrected for multiple comparison by means of a cluster based permutation test (see next section).

1.9. Phase coupling of hippocampal ripples and slow rhythms. For this analysis, the iEEG signal was band-pass filtered for the selected frequency band (delta oscillations [0.5 - 4.5 Hz] and beta [20 - 25 Hz], Butterworth filter, 48 dB/octave). Then, the derived signals were Hilbert

transformed, and the instantaneous phase ($\varphi_{FP}(t)$) value at each ripple amplitude-peak or onset time was used to compute phase distributions using the Rayleigh test (see Statistical analysis). We defined intraelectrode analysis as cross-frequency coupling detected in the same recording channel (i.e., the phase and amplitude frequencies were extracted from the same recording site; e.g., Figure 2), and interelectrode analysis as cross-frequency coupling detected between different recording channels (i.e., reference ripple timestamps and spectral activity were extracted from different recording sites; e.g., Figure 3).

1.10. Statistical analysis. We used two-tailed non-parametric tests. To compare power spectra among cognitive tasks per electrode (Figure 2), we used the Friedman rank test and cluster based permutation test (Maris and Oostenveld, 2007). To evaluate ripple-induced spectrograms, we used the Wilcoxon signed-rank test and cluster based permutation test (see below). To test uniformity of phase distributions, we used the Rayleigh test per electrode and cognitive task, determining the critical Z-value. P values can then be calculated on the basis of the null hypothesis that the phase distribution is uniform. To test modulation in phase distributions among cognitive task, we used the Friedman rank test and post-hoc analyses over Z-value of Rayleigh test of each electrode and cognitive task. In addition, to test for the increase of Z-value in relation to cognitive task, we performed the Page trend test. This test evaluates the hypothesis that a continuous variable (in this case, the Z-value of phase modulation) has the tendency to increase or decrease in relation to a categorical and ordinal variable. For this, we used the cognitive task as ordinal variable in relation to cognitive task (i.e., directed attention task > memory recall task > mind wandering task). The P value was computed by comparing the observed test value (L value) with a random distribution of L values obtained from 10,000 permutations per condition and electrode. This test works as a rank correlation when one of the variables is ordinal (rather than continuous) and the data presents repeated measures (e.g., each electrode was measured in three different conditions). Then, it is possible to compute the correlation coefficient as an extension of the Spearman correlation using *equation 5*:

$$\rho = \frac{12L}{k(n^3 - n)} - \frac{3(n+1)}{(n-1)}$$

where L is the test value, k the number of electrodes and n the number of conditions. Additionally, we performed a partial Spearman correlation controlling for the density of ripples per electrode and condition.

For results that had multiple comparisons, we used a cluster-based permutation test. Thus, clusters of significant areas were defined by pooling neighboring sites (in the frequency spectrum, time-frequency chart or phase-to-amplitude modulation) that showed the same effect ($p < 0.05$). The cluster-level statistics was computed as the sum of the statistics of all sites within the corresponding cluster (e.g.; L value for Page trend test). We evaluated the cluster-level significance under the permutation distribution of the cluster that had the largest cluster-level statistics. The permutation distribution was obtained by randomly permuting the original data (i.e., permuting the label of cognitive task). After each permutation, the original statistical test was computed (i.e., Page trend test), and the cluster-level statistics of the largest cluster resulting was used for the permutation distribution. After 1,000 permutations, the cluster-level significance for each observed cluster was estimated as the proportion of elements of the permutation distribution larger than the cluster-level statistics of the corresponding cluster.

1.11. Software. Processing of anatomical images and electrode localization procedures were performed using the NUTMEG toolbox (Dalal et al., 2004), SPM8 (<http://www.fil.ion.ucl.ac.uk/spm>) and in-house Matlab routines. SPR detection and frequency analysis were performed using in-house Matlab toolbox (available online as LANtoolbox, <http://lantoolbox.wikispaces.com/> and described in detail previously (Billeke et al., 2013)) and Rayleigh test was performed using a circular data toolbox (Berens, 2009). LANtoolbox is largely based on Chronux (www.chronux.org) and FieldTrip (<http://fieldtrip.fcdonders.nl/>) software toolboxes. Supplementary material shows additional detailed data (figures and tables) for every subject in this study.

2. Results

2.1. Two populations of high-frequency oscillations

All recording sites were anatomically identified by MRI after the surgical procedure (Fig. 1A). For all subjects, the epileptic focus was not located in the hippocampus (Table 1) and periods of epileptic discharge were automatically eliminated and confirmed by visual inspection. Ripples were detected by intracranial EEG recordings (iEEG) that were bandpass filtered (80–180 Hz) and thresholded (3.5 S.D.) from the derivative signal of hippocampal recordings (Fig. 1B (Singer and Frank, 2009, Logothetis et al., 2012)). We calculated the amplitude of events in all channels for every subject (Supplementary figures 2-6). In all subjects, the amplitude of events detected in the hippocampus was over 3 S.D. from the overall mean; that is, over the 99th percentile (Fig. 1B), and in most cases, the hippocampal recording sites were the only ones to reach that level. The Morlet complex-wavelet transform was then used to define the frequency spectrum and duration of events (Fig. 1C). Since the number of recording channels located in the hippocampus varied from subject to subject, we considered for subsequent analysis the single channel with the highest-amplitude events per subject (Table 1, Supplementary Figure 7). Visual inspection of the time-resolved wavelet spectrogram of channels with the highest-amplitude events evidenced two frequency ranges where events could be distinguished. One frequency band was compactly centered at around 100 Hz, whereas the other band was wider, and spread around 150 Hz (Fig. 1C). On average, ripples of the slow frequency range had an average frequency and duration of 99.2 ± 8.8 Hz and 66.5 ± 29.6 ms, respectively; consistent with previous reports on humans (Bragin et al., 1995, Axmacher et al., 2008). To better detect events from the fast frequency range, iEEG signals were normalized (Sirota et al., 2008) to compensate for the low amplitude of high frequency signals due to the $1/f$ power law governing the decay of brain electrical signals (Pritchard, 1992, Novikov, 1997). Normalizing the iEEG signal yielded a population of ripples (Fig. 1D) of faster frequency (150 ± 16.4 Hz) and shorter duration (51.4 ± 23.2 ms) than events from the slow frequency range. In order to establish if ripple events were distributed in two different populations, we implemented two independent clustering methods. Indeed, K-means (Fig. 1E) and hierarchical (Fig. 1F) clustering confirmed that ripples could be grouped into two distinct clusters of overlapping events (Supplementary Figures 8-9). These results suggest the existence of two separate populations of ripple episodes in the human hippocampus. Based on these observations and their frequency range, we distinguished slow and fast ripples.

Early studies in animals recognized that the incidence of ripples was strongly dependent upon the ongoing global brain state (O'Keefe and Nadel, 1978, Buzsaki et al., 1983), with further

human studies confirming the observation (Bragin et al., 1999, Le Van Quyen et al., 2010). However, only recently, attention has been paid to the modulation of ripples during waking states with different cognitive content (Kucewicz et al., 2014). Accordingly, we decided to explore the modulation of both types of ripple event during different cognitive tasks, namely: quiet rest, autobiographical memory recall, and directed attention (see Methods). Each cognitive task was associated with a particular power spectrum distribution of hippocampal activity, whose broad shape was similar, yet statistically significant differences could be detected in low-frequency ranges, i.e. delta-theta bands (2-8 Hz, Fig. 2A). In addition, our analysis revealed in all subjects and conditions a prominent peak in the alpha range, consistent with previously reported electrocorticographic patterns in humans (Bahramisharif et al., 2013). Most ripple parameters, including: density, duration, frequency, and amplitude, were not significantly different for the three tested brain tasks (Table 3, Supplementary Figures 10-11).

2.2. Phase modulation of ripple oscillations

Ripples are known to be phase-modulated by slow cortical oscillations (Battaglia et al., 2004, Axmacher et al., 2008, Logothetis et al., 2012, Leonard et al., 2015). Thus, we carried out a nested frequency analysis (Tort et al., 2008, He et al., 2010) in time windows centered in ripple episodes in order to assess inraelectrode phase modulation. We first explored possible modulation effects by cognitive task. Using the Page trend test, we evaluated changes in the phase-to-amplitude modulation related to cognitive task ($qr < arm < da$, see Methods). Indeed, we found that during slow ripples (i.e., modulation in a 200 ms window centered at ripple peaks, subtracting background modulation; see Methods) the phase-to-amplitude modulation increased according to cognitive tasks. Specifically, this increase took place between the phase of delta oscillations (0.5-4.5 Hz) and the amplitude of slow ripples (90-100 Hz), as well as between the phase of beta oscillations (20-25 Hz) and the amplitude of slow ripples (Fig. 2B). Multiple comparisons showed that even though their size was small, clusters were statistically significant. No significant cluster was found for fast ripples. To further confirm these results, we evaluated the two significant clusters found for slow ripples using a mixed linear model. In this model we considered the number of events detected per electrode and task as control regressors. In doing so, we confirmed that statistical significance was preserved only for delta modulation ($t(15) = 2.85$, $p = 0.01$), and not for beta modulation ($t(15) = 2.6$, $p = 0.056$). We then assessed if modulation was specifically related to ripples by exploring the phase distribution of ripple peaks related to the two frequency bands (i.e.; delta and beta oscillations). Accordingly, slow ripples, but not fast ripples, were selectively phase-coupled to delta oscillations in some electrodes, but not to beta oscillations, depending on the cognitive task (Fig. 2C). Interestingly, there was a significant tendency for phase-coupling to increase with cognitive tasks (Page test, Fig. 2C) as slow ripples were less uniformly distributed around delta cycles during directed attention than during quiet rest or autobiographical memory recall (Friedman test, $\chi^2 = 9.17$, $df = 2$, $p = 0.01$; Fig. 2C); a tendency that remained significant when controlled for variations in density between different cognitive tasks (partial Spearman $\rho = 0.35$, $n = 102$, $p = 0.0001$). This observation was specific to delta oscillations, as phase-coupling of ripples with beta oscillations did not change in relation to cognitive tasks (Fig. 2C). Consistent results were found when using a mixed linear model for the phase coupling between slow ripple onset and delta phase ($t(66) = 2.8$, $p = 0.006$; controlled for ripple density).

2.3. Temporal correlation of ripple episodes

Recent animal studies have shown that ripples can be generated locally, but propagate with complex patterns in the septotemporal or temporoseptal direction along the hippocampal axis (Patel et al., 2013). To examine the spatiotemporal organization of ripples in the human hippocampus, we calculated the interelectrode cross-correlation function between ripples detected in the channel with the highest-amplitude events and all other channels located in the hippocampus (Table 2). Thus, we found that there was a significant temporal association of slow ripples across the hippocampus, whereas fast ripples took place randomly and were not temporally coordinated (Fig. 3A-B). In addition, the normalized cross-correlograms showed that the fraction of correlated slow ripples was relatively small (~20%), regardless of the cognitive task. In addition, recent experiments in monkeys have shown that ripples are able to engage and coordinate anatomically distant cortical regions, while subcortical structures are consistently inhibited (Logothetis et al., 2012). Accordingly, we evaluated neural activity patterns associated to slow ripples by constructing spectrograms of hippocampal and neocortical activity (Supplementary Figure 13). When using slow ripples detected in the reference electrode as seed for calculating the interelectrode spectrograms of the other hippocampal electrodes, we found an apparent increase in activity restricted to the slow ripple-frequency band (~100 Hz in frequency, ~50 ms in duration; Fig. 3C). The detected power increase, though statistically significant (Wilcoxon test, FDR < 0.05), was well below criterion to be considered itself as a ripple (3.5 S.D.). That is in accordance with the fact that only 20% of the detected events in the selected electrodes were concomitant with detected events in other hippocampal electrodes (Patel et al., 2013). Interestingly, the statistical significance of power increments in neighboring hippocampal sites was dependent on the cognitive task (Fig. 3C). That is, it was not significant for the condition of quiet rest, but it was significant for the conditions of autobiographical memory recall and directed attention. Furthermore, there was significant correlated activity in electrodes located in the ipsilateral parahippocampal cortex in the same frequency band as slow ripples, specifically for the condition of directed attention (Fig. 3E), suggesting that as cognitive task changed, coordinated neural activity required the cooperation of larger networks in the hippocampo-cortical axis. We then performed the same analysis using fast ripples as seed, and as expected by their low temporal correlation, we detected no significant changes in power in any frequency band of neighboring hippocampal or parahippocampal sites (Wilcoxon test, FDR $p > 0.1$; Fig. 3D, F). We then performed direct comparisons (point-by-point in the time-frequency chart) and no significant difference was detected after multiple comparison corrections (Friedman test and FDR < 0.05). Nevertheless, we explored possible differences in the duration of the modulation of neighboring sites. To this end, we calculated for each electrode the number of sites in the time-frequency chart with significant increases related to ripple detection in the reference channel. For the frequency band of gamma and slow ripples (40 – 120 Hz) we found increases in the number of sites significantly modulated (ipsilateral hippocampus, Friedman test $\chi^2 = 12.93$, $p = 0.001$; post hoc, directed attention > quiet rest, $p = 0.001$; directed attention > autobiographical memory recall, $p = 0.02$. Mixed linear model, directed attention > autobiographical memory recall > quiet rest, $t = 3.47$, $p = 0.001$). This modulation was also significant for ipsilateral parahippocampal electrodes (Friedman test $\chi^2 = 9.12$, $p = 0.01$; post hoc, directed attention > autobiographical memory recall; $p = 0.01$. Mixed linear model, directed attention > autobiographical memory

recall = quiet rest, $t = 2.37$, $p = 0.02$). Thus, slow ripples seem to recruit increasingly larger anatomical territories as cognitive tasks become more demanding, whereas fast ripples remain local.

3. Discussion

Here, we recorded hippocampal activity from human subjects during different cognitive tasks, which were likely associated with distinct brain states. Our results show that there were two distinct populations of ripples that could be distinguished, mostly based on their frequency span. This conclusion was confirmed by two independent clustering methods, which yielded consistent results. Similar results have been recently described, where intracortical recordings in epileptic patients detected different types of high frequency oscillation, which were associated with certain aspects of cognitive processing (Kucewicz et al., 2014). Our results were also consistent with the trend of slower episodes (slow ripples) to be more abundant than faster oscillations (fast ripples), in accordance with the power law governing the decay of brain electrical signals (Pritchard, 1992). We believe that what we call here slow and fast ripples are in fact the same type of activity that Kucewicz et al. 2014 refer to as gamma and ripple events; respectively. Even though we cannot rule out completely the possibility that the events described here are in fact high-amplitude fast-frequency gamma oscillations, we prefer to refer to both kinds of event as ripples, in order to be consistent with various descriptions in humans (Staba et al., 2002, Clemens et al., 2007, Axmacher et al., 2008) and monkeys (Logothetis et al., 2012). In addition, a recent study has quantified hippocampal ripples in human subjects during sleep (Staresina et al., 2015), finding oscillatory events very similar to the ones described here. Our results show that only slow ripples were differentially modulated by cognitive task, whereas fast ripples were not apparently related to network activity. In addition, slow ripples, were seemingly modulated by cognitive task, which is also consistent with high-frequency oscillations been induced by cognitive processing (Kucewicz et al., 2014). Recent work in primates has shown that theta-band bouts can modulate fast gamma frequency events (80-100 Hz) that differ from the modulation of sharp wave ripples by slow rhythms (Leonard et al., 2015). This is consistent with our findings in humans, where modulation of high frequency oscillations is task-dependent. Importantly, what we call fast ripples are not to be confused with much faster oscillations (250-500 Hz) occurring in close proximity to the epileptic focus and related to pathological processes (Bragin et al., 1999, Staba et al., 2002, Foffani et al., 2007). Indeed, fast ripples reported here were of much lower frequency (100-150 Hz), and were recorded only in sites far away from the epileptic focus. Several studies have provided causal evidence for the relevance of ripples in memory consolidation, during both sleep (Girardeau et al., 2009, Ego-Stengel and Wilson, 2010) and wakefulness (Jadhav et al., 2012). However, it has not been clearly established the role of ripples during memory recall (Axmacher et al., 2008). Our results suggest that ripples might not be critically implicated in memory retrieval, since neither their density nor their amplitude was modified during autobiographical memory recall, as it would be expected if they were directly related to the process. Accordingly, it has been shown that the density of rhinal, but not hippocampal, ripples was correlated with the number of successfully recalled items learned prior to sleep (Axmacher et al., 2008).

Importantly, our recordings of high frequency activity in the hippocampus failed to detect associated sharp waves, as expected from previous studies. Indeed, sharp waves are always detected in tight association with ripple oscillations across different species (O'Keefe and Nadel, 1978, Bragin et al., 1999, Le Van Quyen et al., 2010, Logothetis et al., 2012). However, some recent studies have also described ripple episodes in the absence of apparent sharp wave ripples (Axmacher et al., 2008, Kucewicz et al., 2014). Interestingly, those studies recorded hippocampal activity with macroelectrodes, similar to the ones used in the present study. Thus, one possibility is that the electrical impedance or geometry of macroelectrodes makes them unsuitable to detect sharp waves in the human brain. Another, non-exclusive option is that recordings were performed in most cases in superficial layers of the hippocampus, in the stratum oriens area, where there can be no obvious sharp wave (Ylinen et al., 1995). Our structural resonance images do not have enough resolution as to properly segment the hippocampus to be certain of the precise electrode location in our experiments.

Regarding the tasks used to assess hippocampal activity, it is important to highlight that even though they did not provide quantitative readouts (particularly quiet rest and autobiographical memory recall), they do satisfy the requirement of establishing contextual conditions that will likely favor different brain states. In fact, after finishing tasks, subjects were interviewed and provided detailed oral feedback of their mental operations (i.e.; thoughts and memories) during tasks. Even if we cannot quantitatively control the degree of memory retrieval or task engagement, the different tasks can be associated with either active (directed attention) or resting (quiet rest and autobiographical memory recall) brain states, considering the terms in a loose and broad sense. Such qualitative difference, though basic in principle, is enough to sustain a reasonable comparison between neural correlates of brain states. This is further supported by the finding of differences specifically in the behavior of slow ripples, and not fast ripples, as it would be expected if brain states were similar. Thus, our results suggest that ripples are temporally and spatially organized, particularly during active behaviors, not only during resting states, when memory consolidation takes place, as has been thoroughly reported in animal studies (Buzsaki et al., 2003, Battaglia et al., 2004, Battaglia et al., 2011, Buzsaki and Silva, 2012).

If ripples exert a role in information transfer and synchronizing distant anatomical regions (Buzsaki and Silva, 2012), our observation is consistent with the hippocampal system being recruited not only during memory tasks in humans, but being also spontaneously active during task-free epochs, as has been previously suggested (Buckner, 2010). We carried out statistical mapping of the neighboring recording sites where ripples were detected. Unexpectedly, we found correlated oscillatory activity in the range of slow ripples propagating anatomically in relation to the cognitive task. Indeed, during quiet rest, slow ripples were not associated with significant oscillatory activity in neighboring hippocampal areas. Instead, during autobiographical memory recall, the subject was engaged in actively remembering and the memory retrieval system was recruited, slow ripples were correlated with significant oscillatory activity in neighboring hippocampal areas. Finally, during directed attention, the subject was actively engaged in a sensory task, attentional networks and working memory system were recruited, slow ripples correlated with significant oscillatory activity in neighboring hippocampal areas and parahippocampal cortex.

Taken together, our results support the idea that ripples engage and coordinate distant cortical regions (Battaglia et al., 2011, Logothetis et al., 2012). However, under the tested conditions such coordination is not exclusively at the service of memory consolidation (Buzsaki, 1989). Instead, it probably serves to synchronize the collective behavior of distant neuronal populations, each one related to distinct aspects of the cognitive task. Supporting this idea, we found that depending on the task, the phase-coupling of slow ripples to delta oscillations also increased, suggesting that synchrony between hippocampal activity and cortical slow rhythms might be instrumental to coordinate attentional networks. Indeed, there is evidence that transient coupling between low- and high-frequency brain rhythms coordinates activity in distributed cortical areas, providing a mechanism for effective communication during cognitive processing in humans (Canolty et al., 2006). Delta oscillations are mostly abundant during slow wave sleep (Steriade, 2000), during which they modulate the occurrence of ripples (Sirota et al., 2003, Battaglia et al., 2004, Axmacher et al., 2008); yet, delta waves have also been reported in lightly anesthetized and awake resting monkeys, phase-modulating the occurrence of ripples (Logothetis et al., 2012). In addition, phase-coupling during attentionally-demanding situations might be operational for action planning (Singer, 2009), since synchrony of ripples with cortical activity could optimize connectivity; for example, between the hippocampus and medial prefrontal cortex (Siapas et al., 2005, Peyrache et al., 2009).

Another proposed mechanism for the hippocampus to entrain neighboring cortical areas is the cross-frequency dialogue between brain regions, in which high-power (> 3 S.D.) gamma episodes emerge in the cortex during sleep, in a significant number of cases, taking place in a narrow temporal window (100 ms) following hippocampal ripples (Le Van Quyen et al., 2010). It has been suggested that these high-power gamma patterns briefly configure excitable states similar to wakefulness, that are important for consolidation of memory traces acquired during previous awake periods (Le Van Quyen et al., 2010). According to this idea, ripples would propagate memory traces from the hippocampus to the neocortex, which remains in a particularly receptive state during the brief gamma episodes. Our ripple-triggered spectrograms did not show evident increases in gamma-band activity. However, it should be noted that high-power gamma episodes are a small fraction (99th percentile) of the overall gamma-band activity, which coordinates with a small proportion of ripples (10%, (Le Van Quyen et al., 2010). Hence, this specific mechanism of hippocampal-cortical communication is unlikely to be detected by our ripple-triggered spectrograms, and cannot be excluded by our results. Overall, we have found two populations of ripple events in the human hippocampus during waking states. Only one population, slow ripples, seems to be modulated by brain state, supporting the idea that ripples transfer information and coordinate distant anatomical regions, not just in the context of systems consolidation during sleep, but also likely at the service of recruiting cortical regions in waking states to optimize behavioural performance during cognitive demanding situations.

4. References

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Legends

Figure 1. Two distinct populations of ripple episodes in the human hippocampus. **(a)**, Magnetic resonance imaging from a subject (S1) showing the position of the recording channel shown in C in the sagittal (left), coronal (middle), and horizontal (right) planes. **(b)**, Amplitude of events detected in all recordings channels across the brain. Dotted line shows 3 S.D. of amplitude (99.73th percentile). Note only channels located in the hippocampus (red) consistently reach the 3 S.D. threshold. **(c)**, Broadband (0.1-512 Hz) iEEG recording (top panel) and filtered signals (slow ripples and fast ripples, medium panels) from the human hippocampus at quiet rest showing examples of ripples (asterisks); and complex Morlet-wavelet spectrogram for the same recording period (bottom panel). **(d)**, Overall average of complex Morlet-wavelet spectrograms per cluster and average filtered signals. **(e)**, K-means automatic clustering (left panel) for ripples. Average spectra (right panel) for all ripples ($n = 134$, dotted line; note shoulder at high frequency), slow ripples (blue, $n = 87$) and fast ripples (red, $n = 47$). **(f)**, Hierarchical dendrogram for ripples shows two clusters. All data from subject S1. Data for the other experimental subjects is shown in Supplementary Material.

Figure 2. Temporal density and intraelectrode phase-coupling of ripple episodes varies during distinct cognitive tasks. **(a)**, Power spectral density of iEEG recorded during different cognitive tasks (Friedman test, corrected: FDR $p < 0.05$). Each spectrum is a grand average ($n = 34$ electrodes, 6 patients). Note peak in the alpha band (~ 11 Hz). **(b)**, Intraelectrode phase-amplitude co-modulation during slow ripple time windows (200 ms) with background modulation subtracted. Masking depicts background modulation (Spearman correlation and cluster based-permutation test, $p > 0.05$). Highlighted areas represent clusters of significant

differences (Cluster based permutation test). Data is from all cognitive tasks combined. No cluster persists after correction for multiple comparisons for time windows containing fast ripples (**c**), Phase modulation of ripple episodes during delta waves (left) and beta oscillations (right). Asterisks represent statistical differences ($p = 0.02$) between conditions (Friedman test and post hoc analysis). Error bars represent 95%-confidence intervals. qr, quiet rest; amr, autobiographical memory recall; da, directed attention.

Figure 3. Interelectrode temporal correlation of ripple episodes and associated activity in the parahippocampal cortex during different cognitive tasks. (**a, b**), Interelectrode linear cross-correlation function between ripples detected in channels with highest-amplitude events (reference) and all other neighboring channels located in the hippocampus during quiet rest. Note that only slow ripples are coordinated. Similar distributions were found for the other cognitive tasks. Blue, cross-correlation function for ripple episodes; red, shuffling. (**c**), Interelectrode average spectrograms from the ipsilateral hippocampus triggered by slow ripples detected in the hippocampal recording channel with the highest-amplitude events (reference, time 0) during different cognitive tasks. (**d**), Average spectrogram from the ipsilateral parahippocampal cortex triggered by slow ripples detected in the hippocampal recording channel with the highest amplitude events ($n = 11$ electrodes, 4 subjects). Note that power increase was significant only during directed attention. Transparent mask covers regions that did not reach the significance criterion ($n = 26$ electrodes, 6 subjects; Wilcoxon test, false discovery rate < 0.05). (**e, f**), Power changes were not significant for fast ripples in any cognitive task.

Tables

Subject	Age	Gender	Epileptogenic zone	electrode	X	Y	Z	Hippocampal recording electrodes
S1	21	F	Right TPJ	l'2	-19	-43	-4	c'5 ,c'6, l'2, a4, b4, c2, c3
S2	26	F	Left MTG	c'4	-32	-35	-7	c'2, c'3, c'4, b'2, b'3, b'4, b'5
S3	53	M	Right TOPJ	h2	34	-29	10	u3, u2, h2, h3, h4
S4	24	M	Right BTL	b2	25	-15	-15	b2 , b3, c2, c3, c4
S5	23	F	Left MTG	b'2	-20	-19	-12	b'2 , b'3, c'2, c'3
S6	41	F	Left BTL	b4	32	17	20	b2, b4 , b'2, b'4, c'2, c'4

Table 1. Basic patient information and MNI coordinates of hippocampal recording sites per subject. Black font depicts channels with the highest-amplitude events and used for ripples extraction and reference in cross-correlations and time-resolved spectrograms. Coordinates are expressed in millimeters in the MNI coordinate system (Collins et al., 1994). F, female; M, male; TPJ, temporo-parietal junction; MTG, middle temporal gyrus; TOPJ, temporo-occipital-parietal junction; BTL, basal temporal lobe. The name of each electrode is constituted by a letter followed by a number. The choice of the letter of each electrode corresponds to clinical criteria. Usually, the letter refers to the target region of interest (e.g., h for hippocampus, i for insula, f for fusiform gyrus, a for amygdala, etc.). In the case of numbers, they represent the

relative position along the recording sites. Smaller numbers indicate deeper locations from the cortex surface (i.e., the deepest electrode is always number 1). An apostrophe ('), when present, indicates electrode location in the left hemisphere. Otherwise, electrodes are located in the right hemisphere.

	Hippocampus					Parahippocampus				
subject	electrode	X	Y	Z	distance (mm)	electrode	X	Y	Z	distance (mm)
S1	a4	40.00	6.02	-38.99	85.27	e'2	27.08	-21.92	-26.20	56.22
	b2	30.20	-14.66	-23.29	60.96	e'4	33.04	-22.03	-25.96	61.16
	b4	38.08	-14.77	-22.98	67.44					
	b6	45.86	-14.94	-22.56	74.07					
	c2	27.95	-27.38	-10.47	50.92					
	c'2	-19.42	-35.14	-7.52	9.35					
	c'5	-31.74	-34.87	-8.41	15.50					
	c'7	-39.87	-34.68	-8.92	22.55					
	l'2*	-19.82	-43.94	-4.39	0					
S2	b'2	-25.35	-14.87	-15.44	23.33	e'2	-24.11	-16.55	-28.29	29.59
	b'4	-33.38	-15.20	-16.18	22.11	e'4	-29.11	-16.63	-28.44	28.59
	c'2	-24.82	-34.86	-7.17	7.98					
	c'4*	-32.79	-35.38	-7.45	0					
S3	u2	37.41	-30.27	-10.96	2.69					
	h2*	34.82	-29.54	-10.48	0					
	h4	42.22	-29.42	-9.34	7.58					
S4	b2*	25.36	-15.68	-15.29	0	et2	21	-16	-22.10	7.88
	c2	24.29	-32.82	-6.82	19.15	l2	25.63	-43.75	-4.26	30.16
	c4	31.97	-32.58	-6.48	20.18	l4	33.29	-43.71	-4.19	31.18
S5	b'2*	-20.37	-19.16	-12.39	0	j'2	-25	-23	-22	11.18
	c'2	-20.07	-32.40	-1.90	16.90	j'4	-33	-23	-22	16.48
S6	b2	28.00	-17.00	-20.00	61.14	et2	-20	-8.1	-33	22.81
	b'2	-25.00	-16.00	-16.00	8.00	et4	-28	-7.4	-33	19.70
	b4	36.00	-17.00	-19.00	69.07					
	b'4*	-33.00	-16.00	-16.00	0					
	c'2	-28.00	-31.00	-7.70	17.86					
	c'4	-36.00	-31.00	-8.20	17.17					

Table 2. Detailed electrode information and MNI coordinates of hippocampal (Hipp.) and parahippocampal (Parahipp.) recording sites per subject. Asterisk (*) depicts channels with the highest-amplitude events and used for ripples extraction and reference in cross-correlations, time-resolved spectrograms and propagation analysis. In all cases, bipolar signals were used and obtained by subtracting the voltage recording in one channel from the immediately previous channel (located 3 mm away). For example, for l'2, the voltage signal was obtained by l'2-l'1. Coordinates (X, Y, Z) are expressed in millimeters in the MNI coordinate system. Distance refers to the linear distance calculated from the electrode to the seed channel.

	Cognitive task			Friedman test		Mixed linear model	
	qr	amr	da	chi	p	t	p
Slow ripples							
Amplitude (z)	2.56 ± 0.13	2.41 ± 0.06	2.56 ± 0.12	1.58	0.45	<1.44	>0.15
Density (s ⁻¹)	0.252 ± 0.010	0.244 ± 0.005	0.245 ± 0.007	3.48	0.17	<0.91	>0.36
Frequency (Hz)	94.8 ± 1.9	96.5 ± 0.7	94.1 ± 2.3	0.4	0.81	<0.66	>0.5
Duration (ms)	66.9 ± 1.2	66.2 ± 1.3	66.4 ± 1.09	3.82	0.14	<1.29	>0.2
Fast ripples							
Amplitude (z)	3.11 ± 0.14	5.54 ± 2.38	3.29 ± 0.22	0.4	0.81	<1.33	>0.19
Density (s ⁻¹)	0.124 ± 0.007	0.109 ± 0.004	0.111 ± 0.007	4.8	0.09	<1.83	>0.07
Frequency (Hz)	147.3 ± 1.6	147.2 ± 1.4	146.8 ± 1.19	1.42	0.48	<0.17	>0.86
Duration (ms)	50.1 ± 1.1	49.1 ± 1.44	48.2 ± 0.6	1.01	0.58	<1.76	>0.08

Table 3. Ripple parameters evaluated in different cognitive tasks.





