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Ripples in the medial temporal lobe are relevant for human memory consolidation

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High-frequency oscillations (ripples) have been described in the hippocampus and rhinal cortex of both animals and human subjects and have been linked to replay and consolidation of previously acquired information. More specifically, studies in rodents suggested that ripples are generated in the hippocampus and are then transferred into the rhinal cortex, and that they occur predominantly during negative half waves of neocortical slow oscillations. Recordings in human epilepsy patients used either microelectrodes or foramen ovale electrodes; it is thus unclear whether macroelectrodes, which are routinely used for pre-surgical investigations, allow the recording of ripples as well. Furthermore, no direct link between ripples and behavioural performance has yet been established. Here, we recorded intracranial electroencephalogram with macroelectrodes from the hippocampus and rhinal cortex contralateral to the seizure onset zone in 11 epilepsy patients during a memory consolidation task while they were having an afternoon 'nap', i.e. a sleep period of ~1 h duration. We found that ripples could reliably be detected both in the hippocampus and in the rhinal cortex and had a similar frequency composition to events recorded previously with microelectrodes in humans. Results from cross-correlation analysis revealed that hippocampal events were closely locked to rhinal events and were consistent with findings on transmission of ripples from the hippocampus into the rhinal cortex. Furthermore, hippocampal ripples were significantly locked to the phase of hippocampal delta band activity, which might provide a mechanism for the reported phase-locking to neocortical slow oscillations. Ripples occurred with the highest incidence during periods when subjects lay awake during the nap time. Finally, we found that the number of rhinal, but not hippocampal, ripples was correlated with the number of successfully recalled items (post-nap) learned prior to sleep. These data confirm previous recordings in animals and humans, but move beyond them in several respects: they are the first recordings of ripples in humans during a cognitive task and suggest that ripples are indeed related to behavioural performance; furthermore, they propose a mechanism for phase-locking of ripples to neocortical slow waves via phase coupling to hippocampal delta activity; finally, they show that ripples can be recorded reliably with standard macroelectrodes in the human brain.

Keywords: hippocampus; ripples; intracranial EEG; memory consolidation; phase-locking

Abbreviations: EEG = electroencephalogram; REM = rapid eye movement

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Introduction

The formation of declarative memories has been suggested to occur in two subsequent steps. While initial encoding involves the formation of transient representations via fast synaptic plasticity in the hippocampus, consolidation refers to the transfer of information into the neocortex, where more stable networks are built (Buzsaki, 1989; Hasselmo, 1999; Stickgold *et al.*, 2001; Fries *et al.*, 2003; Wiltgen *et al.*, 2004). These two steps correlate with distinct patterns of neural activity in the hippocampus. Initial encoding, which occurs during periods of exploratory behaviour when the organism receives intense sensory input, is accompanied by prominent theta (4–8 Hz; Grastyan *et al.*, 1959; Buzsaki, 2002) and gamma (around 40 Hz; Bragin *et al.*, 1995) oscillations. Consolidation, on the other hand, depends on an offline processing mode during which hippocampal neurons fire in large asynchronous population bursts called sharp waves superimposed by high frequency 'ripple' oscillations (Wilson and McNaughton, 1994; Skaggs and McNaughton, 1996; Louie and Wilson, 2001; Lee and

Wilson, 2002; Foster and Wilson, 2006). In humans, consolidation of declarative memories appears to be closely related to slow-wave sleep (Gais and Born, 2004; Gais *et al.*, 2006).

The initial observations of hippocampal ripples have been obtained in animals, both *in vivo* (Buzsaki *et al.*, 1992; Chrobak and Buzsaki, 1996) and *in vitro* (Draguhn *et al.*, 1998; Maier *et al.*, 2003). Data from the human hippocampus are difficult to assess because of its deep location and specific field properties that do not allow to record hippocampal electroencephalogram (EEG) via scalp recordings. However, epilepsy patients with intracranial electrodes implanted for pre-surgical diagnostics offer the unique opportunity to study neural activity from within the human hippocampus. Using microelectrodes with a diameter of 40 μm, ripples with a maximum power between 80 Hz and 140 Hz were detected in the human hippocampus and rhinal cortex (Bragin *et al.*, 1999*a*, *b*; Staba *et al.*, 2002*a*). These events have been differentiated from even faster oscillations between 250 Hz and 500 Hz termed 'fast ripples', which occur in close proximity to the epileptic focus and appear to be related to pathological processes (Bragin *et al.*, 1999*a*, *b*; Staba *et al.*, 2002*a*; Foffani *et al.*, 2007). However, none of these recordings were conducted during a memory consolidation experiment; thus, a direct link with a behavioural measure has not yet been tested.

In this study, we recorded intracranial EEG data from the rhinal cortex and hippocampus of epilepsy patients contralateral to the seizure onset zone while they had an afternoon 'nap' of 1 h duration. In a previous paper, we investigated the impact of this nap on memory consolidation and on event-related potentials during retrieval of information that was acquired either before or after the nap (Axmacher *et al.*, 2008). We found that memory consolidation occurred both during sleep and waking states, but that sleep facilitated subsequent learning of new information, consistent with recent findings in animals (Foster and Wilson, 2006; O'Neill *et al.*, 2006) and humans (Peigneux *et al.*, 2006; Yoo *et al.*, 2007). Here, we analysed ripple-like high-frequency bursts within the hippocampus and rhinal cortex during the nap. In particular, we addressed five specific questions. First, we analysed the frequency composition of ripples recorded with macroelectrodes (with a diameter of 1.3 mm) in the human rhinal cortex and hippocampus. We wondered whether ripple-related activity is confined to the high frequency range, as observed in animals, or occurs in a broader frequency band, and whether it would differ between the two structures. Second, we investigated the temporal relationship of rhinal and hippocampal ripples. This has not been done before in humans; *in vivo* recordings in rats suggest that ripples occur first in the hippocampus and are then transferred to the entorhinal cortex (Chrobak and Buzsaki, 1996).

Third, we calculated whether the probability of hippocampal ripples was modulated by the phase of delta band activity in the hippocampus. Such a temporal interaction of hippocampal ripples and delta band activity might be relevant for the coupling of ripples with neocortical activity, which is related to information transfer between hippocampus and neocortex during memory consolidation (Sirota *et al.*, 2003; Isomura *et al.*, 2006; Mölle *et al.*, 2006). Fourth, we wondered whether ripples are indeed restricted to periods of sleep, as suggested by findings specifically linking sleep and memory consolidation, or whether they occur during awake resting states as well, which would be consistent with our previous results that consolidation may occur similarly during sleep and waking state (Axmacher *et al.*, 2008). Finally, we correlated the number of rhinal and hippocampal ripples with a behavioural measure of memory consolidation, the number of correctly recollected items learned prior to sleep.

Materials and Methods

Subjects and paradigm

Eleven patients with pharmacoresistant temporal lobe epilepsy (five women; mean age ± SD: 36.8 ± 10.6 years) participated in the study. No seizure occurred within 24 h before the experiment. Recordings were performed from 2005 to 2006 at the Department of Epileptology, University of Bonn, Germany. All patients had bilateral medial temporal depth electrodes that were inserted for diagnostic purposes using a computed tomography-based stereotactic insertion technique (Van Roost *et al.*, 1998). The location of electrode contacts was ascertained by MRI in each patient and was classified as either hippocampal or rhinal or otherwise. Since our methods cannot clearly separate perirhinal and entorhinal generators, we use the term rhinal cortex without indicating an integrated rhinal processing stage. On average, patients had 2.2 ± 1.0 rhinal and 5.6 ± 1.1 hippocampal contacts (mean ± SD). The study was approved by the local medical ethics committee, and all patients gave written informed consent.

During the nap, which was arranged after lunch, subjects lay on a bed in an electrically shielded, sound and light attenuated room for 60 min. During this period, we obtained polysomnographic recordings consisting of scalp EEG at position Cz, as well as measurements of horizontal and vertical eye movements, electrocardiograms and facial electromyograms. Sleep staging based on the recordings of scalp EEG at position Cz was performed visually for 20 s epochs according to the criteria of Rechtschaffen and Kales (1968). During this time, patients were asleep (at least stage 1) for 47.3 ± 19.2 min (mean ± SD) and lay awake for additional 14.7 ± 10.1 min. Before the nap, subjects were presented 80 pictures of landscapes and houses (presentation time: 1200 ms; interval between presentations: 1800 ± 200 ms). Item processing was monitored by asking subjects to distinguish buildings and landscapes via button press. Fifteen minutes after sleep, a second learning session of the same length followed, in which 80 novel items were shown. After a break of 15 min, subjects were presented all images from the two learning sessions together with 80 randomly intermixed new images and were asked to indicate whether they had seen these items before (mean recognition rate, i.e. hits-false alarms: 13.1 ± 2.3; mean ± SEM).

Recording and analyses

Depth EEG was referenced to linked mastoids, recorded at a sampling rate of 1000 Hz, and band-pass filtered [0.01 Hz (6 dB/octave) to 300 Hz (12 dB/octave)]. From the contralateral (non-focal) electrode in each patient, we analysed data from the rhinal contact with the maximal anterior medial temporal N400 amplitude (between 200 ms and 600 ms) and the hippocampal contact with the maximal late positive potential (between 400 ms and 1500 ms). These selection criteria were chosen because the rhinal anterior medial temporal N400 potential and the hippocampal late positive potential are correlated with successful memory formation (Fernandez *et al.*, 1999). Data were analysed using the Vision analyzer software (Brain Products, Gilching, Germany) and MATLAB (The Mathworks, Natick, MA).

The following criteria were used for the selection of artefact and ripple events. Artefacts were identified if they either had a gradient of >30 μV/ms, or if the amplitude in a 1 s interval exceeded 750 μV. Data from −250 ms to +250 ms around these events were excluded. Ripples were detected by filtering the data between 80 Hz and 140 Hz (Butterworth filter, 48 dB/octave) and then selecting all events, which exceeded an amplitude of 20 μV in 12.5 ms time windows contiguously lasting for at least 25 ms length (corresponding to a minimum duration of 2 cycles for oscillations of 80 Hz). Application of these criteria yielded clearly visible bursts of high-frequency activity reminiscent of previous data from animals (Buzsaki *et al.*, 1992; Chrobak and Buzsaki, 1996; Draguhn *et al.*, 1998; Maier *et al.*, 2003) and humans (Bragin *et al.*, 1999*a*, *b*; Staba *et al.*, 2002*a*; Clemens *et al.*, 2007) (see Fig. 2 for hippocampal events and Fig. 3 for rhinal events).

Power values were obtained by convolving the signal with a complex Morlet wavelet and extracting the absolute values of the convolved signal. Time-frequency data ranging from 2 Hz to 200 Hz were extracted (2 Hz steps), and 1000 ms at the start and end of the epochs (epoch length: 1000 ms) were discarded to avoid edge effects. Data were baseline corrected (baseline ranging from −200 ms to 0 ms) and transformed into dB for graphical depiction.

Cross correlations between rhinal and hippocampal time-series were calculated based on the convolution of delta-pulses indicating the ripple onset positions with Gaussians (25 ms SD). To calculate the significance threshold of the cross correlation function, we randomly permutated the inter-event intervals gained from the empirical inter-event interval distribution in each patient. This was done to maintain higher order statistical properties of this distribution such as its variance and skewness. Hundred permutations were obtained for each patient, convolved with a Gaussian (25 ms SD), and processed like the empirical data.

The phase relationship between hippocampal delta band activity and ripples was calculated as follows: First, we filtered the hippocampal EEG at 0.5–4 Hz (delta) (Butterworth filter, 48 dB/octave). Then, data were Hilbert-transformed, phase values were extracted and statistics were calculated based on the phase angles at which ripples occurred in each patient. This was performed by transforming the phase values φ into complex vectors $\exp(i \cdot \varphi)$ for each trial and by averaging these vectors across trials. The angle of the resulting vector is the average phase value at which ripples occurred. The length or absolute value of the vector quantifies the variance of the phases (circular variance) and equals 1 if all phases are equal and 0 if phases are exactly cancelling out each other (Mardia, 1972). The resulting value was compared with surrogate data obtained by permuting the inter-event interval distribution (as for the significance threshold of the cross correlations). One hundred permutations were run for each patient and processed like the empirical value. In each patient, *z*-values were computed based on the comparison of the empirical values to the distribution of the surrogate data. On a group level, these *z*-values were compared with 0 using two-tailed *t*-tests. Statistics were calculated using SPSS (SPSS Inc., Chicago, Illinois), and *P*-values in the analysis of variances were Huynh-Feldt corrected for inhomogeneities of covariance when necessary (Huynh and Feldt, 1976).

To test whether hippocampal-rhinal coupling of ripples depended on the phase or power of hippocampal delta band activity, we first extracted the phase and power of Hilbert-transformed hippocampal EEG filtered in the delta range at those points in time where hippocampal ripples occurred. We then determined the delay to the next rhinal event and compared phase and power values for various short (<10, <20, . . . , <100 ms) and long (>10, >20, . . . , >100 ms) delays. We then tested whether the distributions for the various short delays differed from a uniform distribution (Rayleigh's test of non-uniformity; Oriana software; Kovach Computing Services, Pentraeth, UK). We also compared the variance of phase distributions for these two groups (e.g. <20 ms delays versus >20 ms delays) by calculating the absolute value of the average phase after transformation into complex space (see above; equal numbers of trials were compared by randomly drawing phase values from the distribution, which contained a larger number of values). This resulted in two values for each subject and each delay (corresponding to circular variance), which were then compared by paired *t*-tests (after Fisher-*z*-transformation). Power values for short and long delays were compared using paired *t*-tests. All angles are indicated in the range between 0° and 360°.

Results

Based on our criteria of 'ripple' selection (see Methods section), we observed 1.07 ± 1.34 (mean ± SD) events/min in the rhinal cortex and 1.90 ± 1.64 events/min in the hippocampus during the naps. During the entire session, we detected between 2 and 312 events in the hippocampus (median 93) and between 2 and 199 events in the rhinal cortex (median 21). These events were at least 32 ms (hippocampus) or 35 ms (rhinal cortex) apart. Figure 1 displays inter-event interval distributions in both structures. This figure shows a peak in the distribution of intervals between 100 ms and 200 ms. Typical examples of individual ripple events from the hippocampus of three different patients are depicted in Fig. 2A, together with their frequency composition. In most patients, the events were due to a circumscribed power increase in the frequency range between 80 Hz and 120 Hz and not due to a broad-band power increase. Averages across all hippocampal events in the same patients are depicted in Fig. 2B, and a grand average across all 11 patients is shown in Fig. 2C. These time-frequency decompositions of unfiltered EEG activity confirm that ripple frequency is restricted to the high frequency range between 80 Hz and 120 Hz. Finally, we calculated the grand average of the unfiltered EEG activity

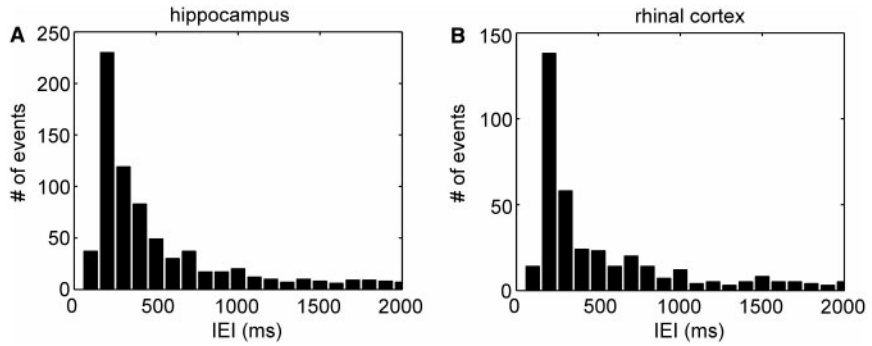

Fig. 1 Inter-event interval distribution of ripples. Distributions both in the (**A**) hippocampus and (**B**) rhinal cortex peaked at intervals between 100 ms and 200 ms.

triggered to the ripple events across all patients (Fig. 2D). While activity before and after the event mostly cancelled out due to averaging across variable phases, there was a clearly visible oscillation with a full-cycle duration of about 300 ms around ripple onset. This component exhibited first a positive deflection starting at 35 ms prior to ripple onset and peaking at around 0 ms, and then a negative deflection with a maximum at 140 ms. It thus involved a full oscillatory cycle at a frequency of around 3.3 Hz with a phase of about 0° (cosine) at the time of ripple onset; its positive peak resembled the physiological sharp wave, which has been described to occur simultaneously with ripples in rodent recordings (Chrobak and Buzsaki, 1996; Maier *et al.*, 2003), even though significantly slower (duration of a half-wave in rodents: around 70 ms).

Individual events from the rhinal cortex looked very similar to hippocampal events (data not shown). Figure 3A depicts averages across trials in the same patients as for the hippocampus, and Fig. 3B shows the grand average across all patients. Again, there was a circumscribed power increase in the high frequency range around 80–120 Hz; however, we observed in addition an enhanced power at around 30 Hz. We also found an EEG oscillation time-locked to the ripple, which, however, displayed a negative peak around ripple onset and had a duration of about 70 ms corresponding to a frequency around 14 Hz, i.e. in the frequency range of sleep spindles (Fig. 3C). In contrast to the hippocampus, this component was not clearly distinct from background fluctuations.

Next, we investigated the temporal relationship of ripples in the hippocampus and rhinal cortex. Figure 4 shows a typical example of the time points at which hippocampal and rhinal events occurred in one patient. Visually, ripples in the two structures occurred at very similar points in time, both on a larger (Fig. 4A) and on a more focused time scale (Fig. 4B). To quantify the temporal relationship of events in both structures, we calculated a cross correlation of the time vectors, which were previously convolved with a Gaussian filter (25 ms SD). Only patients with >10 events in both structures were taken into account for this analysis (this criterion was met by 7 out of the total group of 11 patients). The result is shown in Fig. 4C (the significance threshold was calculated using a bootstrap method based on the empirical inter-event interval distribution; see Methods section). Cross correlation was maximal at a lag of +1 ms; this lag was visible in each individual patient (lag of +1 ms in six patients and +3 ms in one patient). To quantify this effect, we compared between the integral of the cross correlation function for different lags in the positive versus negative direction. We found that for lags <16 ms, the integral between −lag and 0 was significantly ($P<0.05$; Fig. 4D) smaller than the integral between 0 and +lag, suggesting that ripples in the hippocampus occurred significantly earlier than in the rhinal cortex.

Furthermore, we analysed whether hippocampal ripples occurred predominantly during specific phases of hippocampal delta band activity (see Methods section). Only patients with >20 events in the hippocampus were taken into account (resulting in a group of eight patients). Figure 5 depicts distributions of ripples across phase values of delta band activity. Indeed, we observed a significant modulation of ripples by delta phase ($t_7=3.180$; $P<0.05$). The mean delta phase value at which ripples occurred was close to 0° (6.9°), i.e. close to the positive peak, in accordance to our findings for the slow hippocampal oscillation depicted in Fig. 2D.

Does hippocampal-rhinal coupling of ripples depend on the phase or power of hippocampal delta band activity at which a hippocampal ripple occurred? We found that phase distributions at which hippocampal ripples occurred, which were rapidly transferred to the rhinal cortex (delays ranging from <10 ms to <100 ms) were not significantly different from uniform distributions (each $P>0.1$; Rayleigh's tests). A direct comparison between the variance of phase distributions at short and long delays did not reveal significant differences (each $P>0.1$; two-tailed t-tests). Similarly, power values were not different between short and long delays (each $P>0.2$; two-tailed t-tests). Thus, transmission of ripples from the hippocampus to the rhinal cortex does not appear to depend on the phase or power of hippocampal delta band activity during which hippocampal ripples occur.

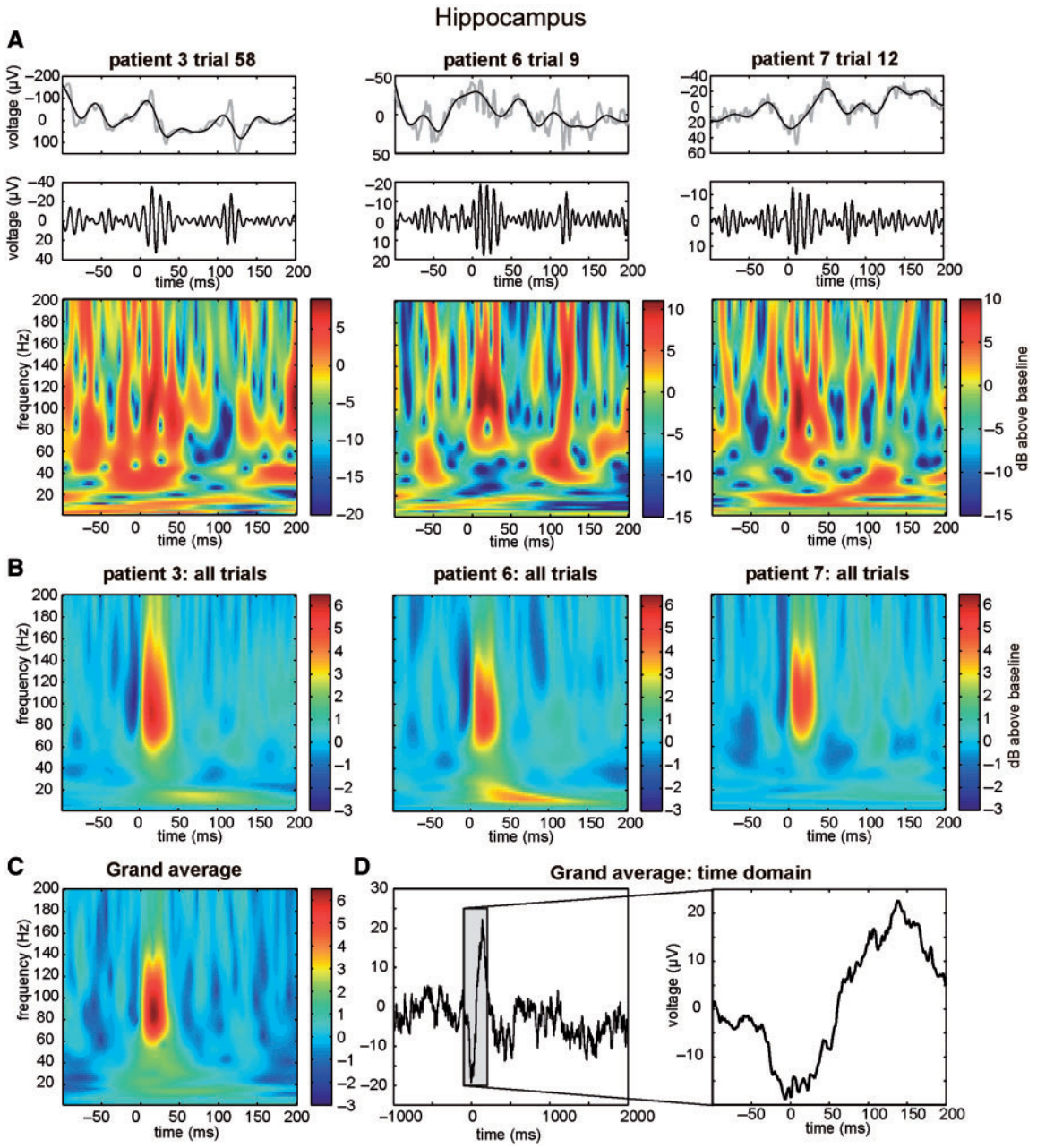

Fig. 2 High frequency oscillations in the human hippocampus. (**A**) Individual events in three patients in the time and frequency domain. Top row: unfiltered raw data (gray trace) and low-pass filtered data (<30 Hz). Middle row: same data filtered from 80 Hz to 140 Hz. Bottom row: time-frequency data. (**B**) Average across all events in the same three patients. (**C**) Grand average of hippocampal ripples across all patients. The maximum power of the selected events is in the range between 80 Hz and 100 Hz. (**D**) Grand average of unfiltered data in the time domain in an extended time scale and in the same time range as above. All data in (**B**–**D**) are unfiltered.

Next, we calculated the proportion of ripples in the different sleep stages. For this analysis, we normalized by the amount of time spent in each sleep stage and afterwards by the total sleep time in each subject. For clarity, Fig. 6A indicates the distribution of sleep stages across patients (please note that similar information is provided as Supplementary Fig. 1 in Axmacher *et al.*, 2008, which is based on the same data set; however, the figure in that previous analysis was normalized by the total amount of time spent asleep, i.e. excluding waking state). This distribution indicates that only a relatively short average time was spent in stage 4 and rapid eye movement (REM) sleep. Moreover, not all patients reached these stages; Fig. 6A also indicates the number of patients who reached the respective sleep stages. We found that the majority of ripples (mean ± SEM: rhinal cortex: 64.6 ± 8.4%; hippocampus: 70.6 ± 8.1%) were detected during waking state, and only a small minority occurred during slow-wave

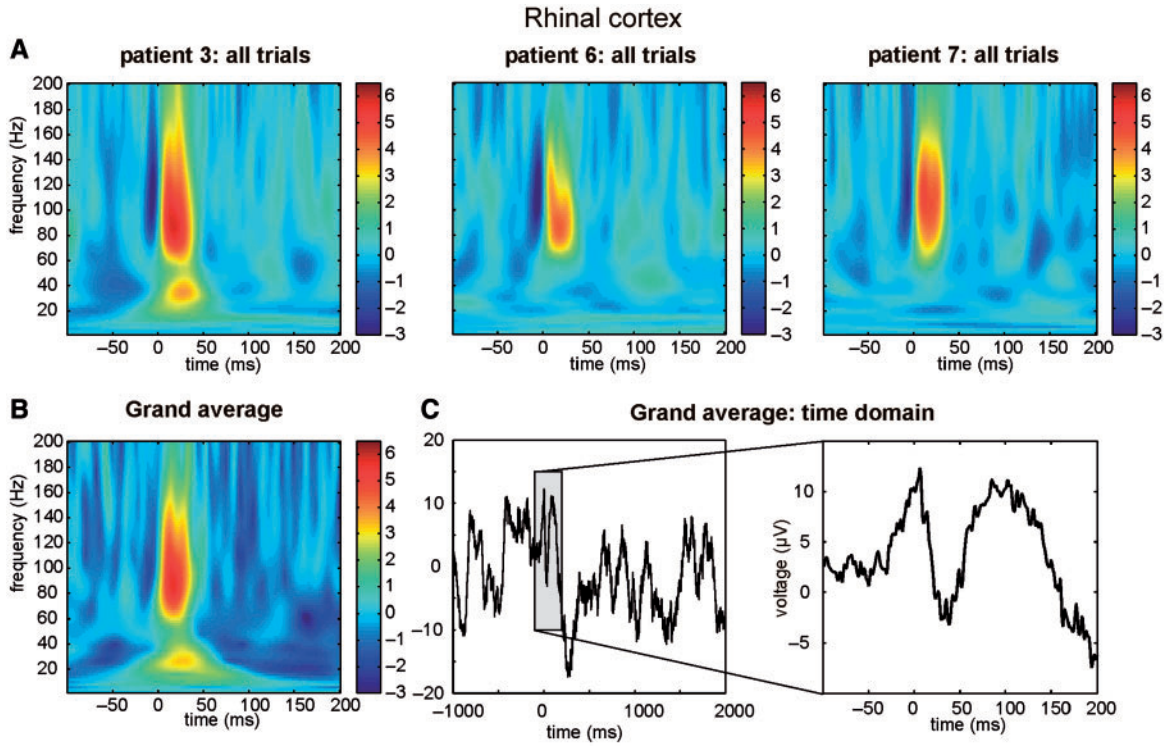

Fig. 3 Ripples in the rhinal cortex. (**A**) Averaged events in the same three patients as depicted in the hippocampus. (**B**) Grand average across all 11 patients. (**C**) Grand average of data in the time domain across all patients. All data are unfiltered.

sleep (Fig. 6B; rhinal cortex: stage 3: $5.7 \pm 2.6\%$; stage 4: 3.0 ± 1.8; hippocampus: stage 3: $3.3 \pm 1.7\%$; stage 4: $1.0 \pm 0.7\%$). A two-way analysis of variance with 'locus' (rhinal cortex versus hippocampus) and 'sleep stage' (awake, stages 1–4, REM) as repeated measures revealed a significant effect of 'sleep stage' ($F_{5,50} = 32.27$; $P < 10^{-5}$; $\varepsilon = 0.45$), but no effect of 'locus' and no interaction.

Finally, we wondered whether the number of rhinal and/or hippocampal ripples was correlated with a behavioural measure of memory consolidation. There was no correlation between the number of hippocampal ripples and memory performance measured as the number of correctly retrieved items learned prior to sleep (Pearson's correlation coefficient $r = 0.08$; $t_9 = 0.25$; $P = 0.81$; Fig. 7A). However, the number of rhinal ripples was significantly correlated with memory performance (Pearson's correlation coefficient $r = 0.67$; $t_9 = 3.67$; $P = 0.005$; Fig. 7B).

Discussion

Using intracranial EEG recordings in the hippocampus and rhinal cortex of 11 epilepsy patients, we investigated the properties of high frequency oscillations (ripples) during an afternoon nap in a memory consolidation experiment. We found that rhinal and hippocampal ripples consisted of bursts with a power maximum around 100 Hz (Figs 2 and 3). Hippocampal events were highly correlated with rhinal ripples (Fig. 4) and the positive peak of hippocampal delta band activity (Fig. 5). Events in both structures occurred predominantly during waking state (Fig. 6). Finally, we found that the incidence of rhinal, but not hippocampal, ripples was highly correlated with a behavioural measure of memory consolidation (Fig. 7).

Electrodes implanted in the peri- and ento-rhinal cortex were collectively termed 'rhinal', although these regions likely support different functional roles: The hippocampus receives its input from the entorhinal cortex, which in turn communicates with the neocortex via the perirhinal cortex. In rats, activity in these two regions is usually relatively independent, giving rise to a low rate of neocortical-hippocampal information transfer (Pelletier *et al.*, 2004). However, ento- and peri-rhinal spikes become synchronized during fear conditioning by inputs from the amygdala (Paz *et al.*, 2006), resulting in an increased transfer of sensory information into the hippocampus. In humans, microelectrode recordings in epilepsy patients revealed a complex pattern of feedforward and feedback processing between ento- and peri-rhinal cortex for instance during word recognition (Halgren *et al.*, 2006). Standard macroelectrode recordings as used in our study, however, do not allow differentiation between activities in these subregions of the parahippocampal cortex. Future studies using microelectrodes are needed to reveal the dynamics of ripple propagation in these regions.

The frequency composition of hippocampal and rhinal ripples recorded with macroelectrodes closely resembled the events observed previously in the human hippocampus using microelectrode recordings (Bragin *et al.*, 1999*a*, *b*;

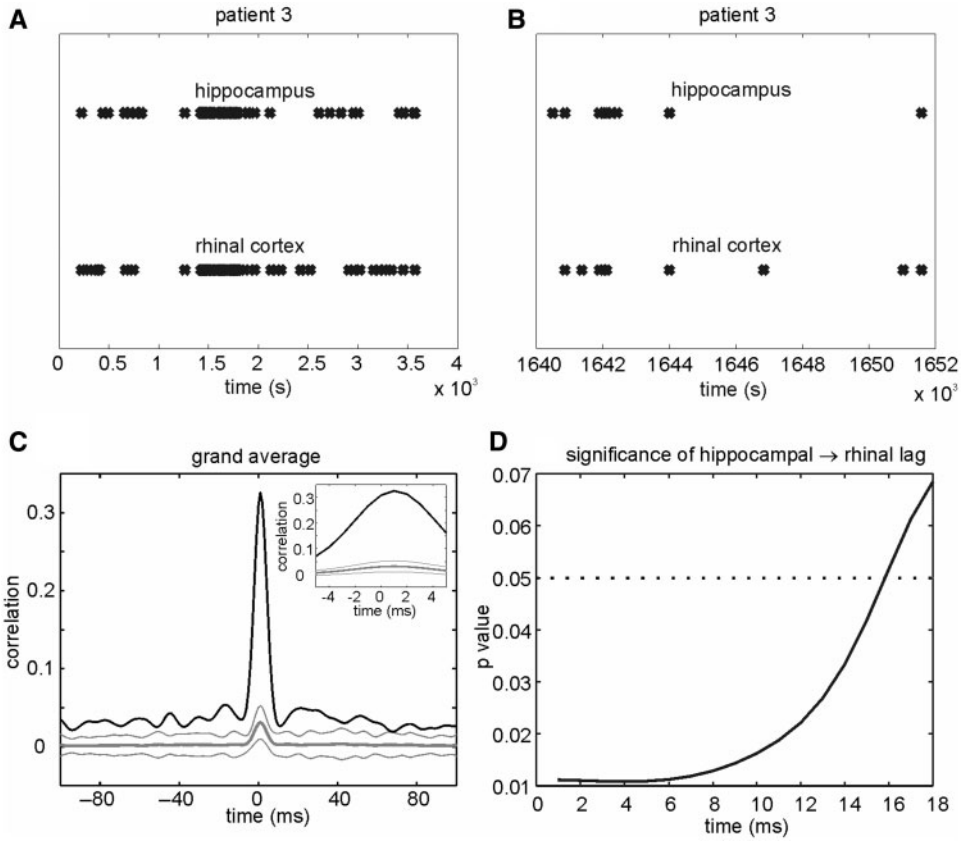

Fig. 4 Cross correlation of hippocampal and rhinal ripples. (**A**) Time points where ripples occurred in the hippocampus and rhinal cortex in one patient. (**B**) Same plot for an extended time scale. (**C**) Averaged cross correlation function across all patients for different lags. The black line indicates the empirical cross correlation function, and the gray lines the mean and standard deviation of the surrogate values. As visible from the small inset, there is a slight (1 ms) lag in the maximal cross correlation, indicating that hippocampal events occurred earlier. (**D**) This effect was statistically significant up to lags of <16 ms, as visible from the comparison of the integrals of the cross correlation function for negative and positive lags.

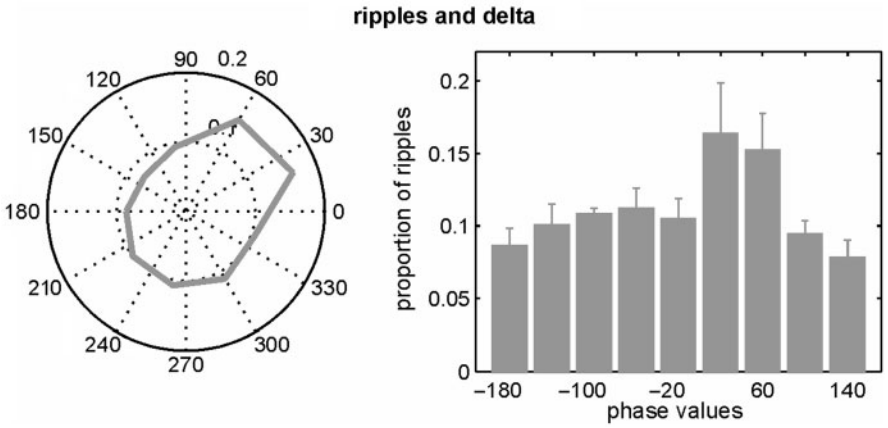

Fig. 5 Cross frequency coupling of hippocampal ripples and hippocampal delta band activity. Activity in the hippocampus was filtered in the delta (0.5–4 Hz) frequency ranges. The phase values at which ripples occurred were extracted and compared with surrogate data obtained by permuting the empirical inter-event interval distributions. The results are depicted both as polar plots and as standard histograms. Ripples were indeed significantly locked to a specific phase of delta band activity ($P < 0.05$).

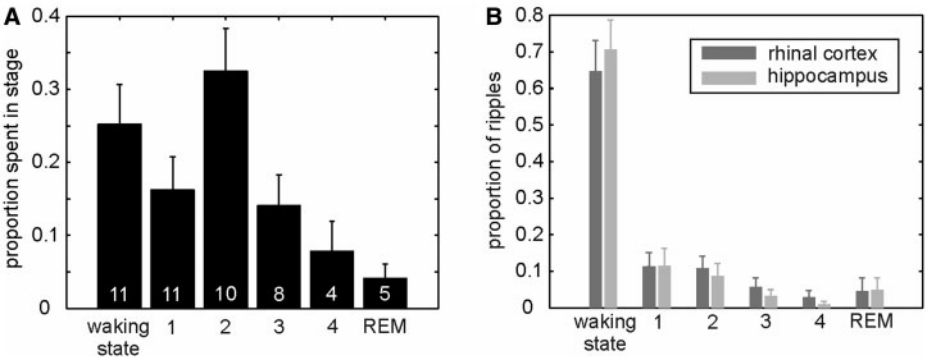

Fig. 6 Occurrence of ripples as a function of the state of vigilance. (**A**) Distribution of waking state and sleep stages in the group of patients (mean and SEM). The numbers indicate how many patients reached the respective stage. (**B**) The majority of all hippocampal ripples occurred during waking state, and only a minority occurred during stages of deep sleep.

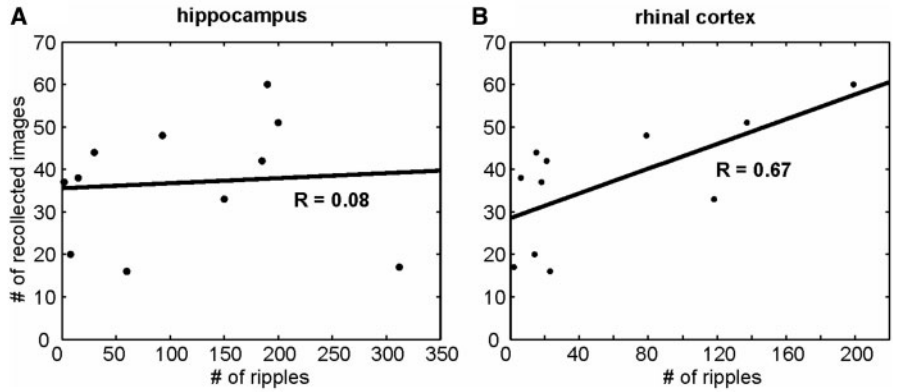

Fig. 7 Correlation of ripples and memory consolidation. (**A**) No correlation between the number of hippocampal ripples and memory consolidation [measured as the number of correctly recollected items (post-nap), which were learned prior to sleep]. (**B**) Highly significant ($P = 0.005$) correlation between the number of rhinal ripples and memory consolidation.

Staba *et al.*, 2002*a*) and in the human parahippocampal cortex using foramen ovale electrodes (Clemens *et al.*, 2007). The frequency content is somewhat lower than the typical frequency range observed in rodents (around 200 Hz; Buzsaki *et al.*, 1992). Several factors might account for this discrepancy. Most importantly, ripple frequency is known to slow down with brain size; for example, the frequency of ripples in macaque monkeys is similar to ripple frequency in human subjects (Skaggs *et al.*, 2007). Furthermore, different electrodes are used in these studies; for example, while Ylinen *et al.* (1995) used 60 µm-thick tungsten wires, Staba and colleagues (2002*b*) utilized platinum-iridium microwires with a diameter of 40 µm and we used macroelectrodes with a diameter of 1.3 mm. In principle, two factors mediate the relationship between electrode size and the frequency of the measured EEG activity. First, larger electrodes likely record activity from larger brain regions, and are thus more sensible to synchronous activity from larger neural assemblies. Synchronization of larger assemblies usually occurs in lower frequency ranges due to longer conduction delays, although there are exceptions such as long-range gamma synchronization (Traub *et al.*, 1996; Rodriguez *et al.*, 1999). Second, the resistance and the frequency-dependent impedance of macro- and micro-electrodes differ (Franks *et al.*, 2005).

Time-locked to ripple onset, we observed a positive component in the hippocampus (Fig. 2D) and a negative potential in the rhinal cortex (Fig. 3C). Previous findings from animals indicate a polarity reversal between the pyramidal cell layer (positive) and the stratum radiatum (negative) of the hippocampal CA1 and CA3 regions (Buzsaki, 1986; Maier *et al.*, 2003), and between layer two (positive) and three (negative) of the entorhinal cortex (Chrobak and Buzsaki, 1996). The macroelectrodes used in our study do not allow to record selectively from a specific layer; thus, a direct comparison with the animal data is not possible. However, our findings of a positive polarity in the hippocampus and a negative polarity in the rhinal cortex suggest that activity from the pyramidal cell layer in the hippocampus and from layer two in the entorhinal cortex exerted a predominant influence. Interestingly, the duration of this oscillation in the hippocampus was much longer than in animals (250 ms versus ~70 ms), possibly due to differences between micro- and macro-electrode recordings

and/or species, whereas it was similar in the rhinal cortex (~70 ms).

Hippocampal and rhinal ripples showed a close temporal correlation (Fig. 4), although almost twice as many hippocampal as rhinal events were observed (113 versus 57). This difference is most likely related to the threshold for events in our detection algorithm. Rhinal ripples occurred after a very small lag of 1 ms after hippocampal events. This effect is probably below the temporal resolution of our recordings (1 kHz). To investigate it further, we calculated the integral of the positive and negative cross correlation function for different lags. We found that for lags of up to 15 ms, the positive integral was significantly larger than the corresponding negative integral (Fig. 4D). The small delay of rhinal and hippocampal ripples is consistent with the idea that events were generated in a third structure and transferred both to the hippocampus and to the rhinal cortex. Alternatively, it might be argued that events recorded in one structure only reflect volume conduction from the other structure. However, this would predict that the number of events in the two structures should be inter-individually correlated, which was not the case ($P = 0.22$). Finally, it is possible that ripples were indeed generated in the hippocampus and transferred to the rhinal cortex. We are not aware of any previous data on the temporal relationship of ripples within the rhinal cortex and the hippocampus in humans. *In vivo* recordings in rats (Chrobak and Buzsaki, 1996) and *in vitro* recordings in mouse brain slices (Maier *et al.*, 2003) indicate that ripples are generated in the hippocampal CA3 region and then transferred to CA1, the subiculum and the entorhinal cortex. These results are qualitatively consistent with our findings, although a larger lag (>10 ms) was reported for rodents, which is similar to the size of the lag up to which we observed an asymmetry of the cross correlation function (15 ms). While we cannot completely explain this discrepancy, it is possible that the macroelectrodes used in our study did not sample the same regions as these animal data; in particular, our hippocampal recordings are likely influenced by activity from the subiculum, where ripples occur with a shorter lag to entorhinal events. Furthermore, species differences also may account for the discrepancy.

Hippocampal ripples were significantly locked to the peak of hippocampal delta band activity (Fig. 5). While the relationship of hippocampal ripples to delta band activity in the same structure has not been investigated so far, previous studies reported phase-locking of ripples to neocortical theta and delta band activity. Neocortical slow oscillations at a frequency <1 Hz correspond to alternating states of enhanced and reduced cortical excitability (up- and down-states) due to membrane potential fluctuations (Steriade *et al.*, 1993). *In vivo* recordings in rats revealed that hippocampal sharp waves were locked to the depolarizing phase of neocortical delta waves/slow rhythm, which is associated with enhanced cortical activity (Sirota *et al.*, 2003; Battaglia *et al.*, 2004; Isomura *et al.*, 2006; Mölle *et al.*, 2006). In humans, a similar relationship was established between parahippocampal ripples and neocortical slow oscillations (Clemens *et al.*, 2007). Here, we propose that the coupling between neocortical slow oscillations and hippocampal ripples results from a phase-locking of ripples to hippocampal delta band activity, which is itself phase-locked to neocortical slow oscillations (Wolansky *et al.*, 2006; Fell *et al.*, 2007).

Delta band activity was investigated by calculating the phase and power of Hilbert-transformed EEG data (after application of a digital filter from 0.5–4 Hz). In principle, results from Hilbert (or wavelet) transforms in specific frequency bands do not indicate underlying oscillatory activity in this range. This difference is important because there appear to be profound species differences for hippocampal activity in this slow frequency range, which are most pronounced in the theta range (data from the rodent hippocampus related to the delta range are still scarce). In contrast to rodents, theta oscillations in humans are difficult to see in the unfiltered EEG, and theta band activity does not exhibit a pronounced peak in the power spectrum; instead, theta band activity appears to occur in brief intermittent bursts (Cantero *et al.*, 2003). On the other hand, several studies have shown that the power of hippocampal theta band activity in humans is correlated with behavioural performance and thus appears to have a functional role (although correlations of course do not imply causality). For instance, hippocampal theta band activity increased during exploration of a virtual town (Caplan *et al.*, 2003; Ekstrom *et al.*, 2005) and during a working memory task (Raghavachari *et al.*, 2001). Importantly, this increase of theta band activity during exploration suggests a similar functional role to theta oscillations in rodents (Buzsaki *et al.*, 1989). Furthermore, it has been suggested that the mapping of ego- to allocentric space representations in rodents and the transformation of episodic into semantic memories in humans depend on a similar computational process, which is implemented by theta band activity (Buzsaki, 2005).

We found that both rhinal and hippocampal ripples occurred predominantly during waking state (Fig. 6), and only a small minority during slow-wave sleep. This might appear surprising as ripples were previously linked to offline information processing during resting state and slow-wave sleep; for example, Staba and colleagues (2004) observed that ripples occurred with a higher rate during non-REM sleep than during waking state. On the other hand, 'waking state' usually corresponds to normal awake behaviour, whereas it meant brief periods during the nap in our study, when subjects lay in a quiet dark room with their eyes closed. Thus, it is more closely related to a resting state than to awake exploratory behaviour. Furthermore, our findings that ripples, which have been linked to hippocampal-neocortical information transfer during consolidation (Sirota *et al.*, 2003; Isomura *et al.*, 2006; Mölle *et al.*, 2006), occur with a high rate during waking state is

consistent with our previous results that consolidation occurs not only during sleep, but also during waking state as well (Axmacher *et al.*, 2008). Further experiments are needed, however, to clarify this issue.

It might appear surprising that all patients were able to fall asleep in a relatively short time interval. All patients had the nap in the early afternoon, where sleep threshold has been shown to be particularly low: For example, a study by Pack and colleagues (1995) investigating the incidence of car accidents due to drivers falling asleep as a function of day time found that crashes occurred predominantly during nighttime and during the early afternoon period. Moreover, we selected those patients for the study who reported to have occasional afternoon naps at home.

Finally, we observed that the frequency of ripples in the rhinal cortex was highly correlated with memory consolidation, measured as the number of correctly recollected items (post-nap) learned prior to sleep (Fig. 7). To our knowledge, this is the first result linking the incidence of ripples and a behavioural measure of memory consolidation. Several explanations are possible to account for this finding. Because we did not record ripples in an additional control nap without prior stimulus presentation, our data do not necessarily support a direct link between ripple incidence and learning. It is possible that rhinal ripples are a trait marker related to general cognitive processing speed, such that subjects with a higher number of rhinal ripples processed items more attentively, were more alert etc. Alternatively, it is possible that ripples are more specifically related to cellular mechanisms of long-term memory formation. This explanation, which is currently still somewhat speculative, would be in accordance with results from animal experiments, which showed that increasing cellular excitability by induction of long-term potentiation enhances both the magnitude of sharp-wave associated ripples in the hippocampus *in vivo* (King *et al.*, 1999) and the incidence of hippocampal ripples *in vitro* (Behrens *et al.*, 2005). Previous studies investigating the mechanism of sleep-related memory consolidation reported that memory consolidation correlated with the incidence of neocortical sleep spindles (Gais *et al.*, 2002), and that ripples and sleep spindles were correlated (Siapas and Wilson, 1998; Sirota *et al.*, 2003; Clemens *et al.*, 2007). Again, a possible correlation of hippocampal delta band activity with neocortical slow oscillations, which are correlated with neocortical sleep spindles (Mölle *et al.*, 2002), might explain the mechanism underlying this effect. In our data, only rhinal, but not hippocampal ripples were correlated with the number of correctly recognized items. We do not have a clear explanation for this result. Together with our finding that rhinal ripples were closely locked to (and perhaps driven by) hippocampal ripples (Fig. 4), one may, however, suggest the following scenario: even though ripples appear to be generated in the hippocampus, only those events that are relevant for the consolidation of previously acquired memories are subsequently transferred to the rhinal cortex. As a result, the number of rhinal, but not hippocampal, ripples is correlated with memory consolidation. This hypothesis would predict that some filter mechanism controls transmission of ripples from the hippocampus into the rhinal cortex, and that the efficiency of this mechanism determines recognition performance. Further experiments are required to test this idea. Most importantly, it would be interesting to record medial temporal ripples both prior to and after learning to investigate whether they are directly related to long-term memory formation.

Taken together, we found that ripples could be recorded with macroelectrodes in the human rhinal cortex and hippocampus and were closely correlated in these two structures. The phase-locking of hippocampal ripples to delta band activity in the same structure might explain phase coupling of ripples to neocortical slow oscillations. Moreover, we report the first direct behavioural evidence that ripples are correlated with memory consolidation. The high incidence of ripples during waking state is consistent with our previous findings that consolidation occurs similarly during sleep and waking state.

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