

Temporal coupling of parahippocampal ripples, sleep spindles and slow oscillations in humans

Zsófia Clemens,¹ Matthias Mölle,² Lóránd Eröss,³ Péter Barsi,⁴ Péter Halász¹ and Jan Born²

¹Department of Neurology, National Institute of Psychiatry and Neurology, Budapest, Hungary, ²Department of Neuroendocrinology, University of Lübeck, Lübeck, Germany, ³National Institute of Neurosurgery, Budapest, Hungary and ⁴MR Research Centre, Semmelweis University, Budapest, Hungary

Correspondence to: Zsófia Clemens, PhD, National Institute of Psychiatry and Neurology, Department of Neurology, H-1021 Budapest, Hűvösvölgyi út 116, Hungary
E-mail: clemens@opni.hu

Ripples are high-frequency oscillation bursts in the mammalian hippocampus mainly present during Non-REM sleep. In rodents they occur in association with sharp waves and are grouped by the cortical slow oscillation such that, in parallel with sleep spindles, ripple activity is suppressed during the hyperpolarized down-state and enhanced during the depolarized up-state. The temporal coupling between slow oscillations, spindles and ripples has been suggested to serve a hippocampo-neocortical dialogue underlying memory consolidation during sleep. Here, we examined whether a similar coupling exists between these oscillatory phenomena in humans. In sleep recordings from seven epileptic patients, scalp-recorded slow oscillations and spindles as well as parahippocampal ripples recorded from foramen ovale electrodes were identified by automatic algorithms. Additionally, ripple and spindle root mean square activity was determined for relevant frequency bands. Ripple density was higher during Non-REM than REM sleep ($P < 0.001$). Ripple activity distinctly decreased time-locked to slow oscillation negative half-waves in the three patients without temporal structural alterations ($P < 0.001$), whereas in the four patients with severe mesiotemporal structural alterations this coupling was obscure. Generally, in the patients ripple activity was increased before spindle peaks and distinctly decreased after the peak ($P < 0.001$). Ripples were consistently associated with interictal spikes suggesting that spike-ripple complexes represent an epileptic transformation of sharp wave-ripple complexes in the epileptic hippocampus. Our findings are consistent with the notion of a hippocampo-to-neocortical information transfer during sleep that is linked to coordinate ripple and spindle activity, and that in the intact temporal lobe is synchronized to cortical slow oscillations.

Keywords: sleep; hippocampus; sharp wave-ripples; slow oscillation; epilepsy

Abbreviations: EOG = electrooculogram; EMG = electromyogram; FIR = finite impulse response; SPW = sharp wave

Received November 22, 2006. Revised May 10, 2007. Accepted May 31, 2007. Advance Access publication July 5, 2007

Introduction

Sleep and especially non-rapid eye movement (Non-REM) sleep is characterized by neuronal electrical oscillations in a wide frequency range that emerge from cortical and thalamocortical networks (Buzsáki and Draguhn, 2004; Steriade, 2006). Amongst these oscillations, the cortical slow oscillation identified by Steriade and colleagues (Steriade *et al.*, 1993a,b) has been proposed as a principal factor in orchestrating faster oscillations binding them into a temporally structured and coordinated network. During the slow oscillation the membrane potential alternates between depolarized and hyperpolarized states corresponding to general facilitation and disfacilitation of cortical neuronal activity. Depolarized up-states, corresponding to surface-positive half-waves of the slow oscillation, are associated with increased sleep spindle activity emerging

from corticothalamic loops, whereas during the hyperpolarized down-states spindle activity is strongly reduced (Steriade *et al.*, 1993a,b; Destexhe *et al.*, 1999; Steriade, 2006). The slow oscillation occurs in the human EEG with a dominant spectral peak of about 0.8 Hz (Achermann and Borbély, 1997) and like in animals it has been shown to efficiently group sleep spindle activity (Mölle *et al.*, 2002).

In animals the cortical slow oscillation exerts a grouping influence also on hippocampal high-frequency activity. Thus during the transition into as well as during up-states, hippocampal high-frequency (100–200 Hz) bursts, i.e. ripples, occur at an increased rate, whereas their frequency is distinctly reduced during the down-state (Sirota *et al.*, 2003; Battaglia *et al.*, 2004; Mölle *et al.*, 2006; Isomura *et al.*, 2006). Ripples originate in the CA1 region of the hippocampus (Csicsvári *et al.*, 1999) and occur in

Table 1 Patient characteristics

Patient	MRI	Seizure onset	Age (yr)	Years of intractable seizures	Sex
1	Right insular alteration	Extratemp.	26	1	F
2	Negative	Extratemp.	34	22	M
3	Negative	Right mesiotemp.	33	2	M
4	Right HS	Bilateral mesiotemp.	49	27	M
5	Left HS	Left mesiotemp.	48	40	F
6	Right HS	Right mesiotemp.	40	23	F
7	Bilateral temp. alterations	Bilateral mesiotemp.	41	40	F

Note: HS, hippocampal sclerosis. Note, structural alterations were diagnosed by visual inspection of MRI scans alone, as in most cases histological probes were not available.

association with sharp waves emerging from CA3 (Buzsáki, 1986). Hippocampal stimulation that induces long-term potentiation, a neurophysiological correlate of learning, concurrently facilitates the generation of sharp wave–ripples in CA3 (Behrens *et al.*, 2005). Sharp wave–ripple complexes were revealed to be also temporally coupled to sleep spindles. Importantly, the coordinate spindle–ripple events have been suggested to provide a mechanism for information transfer between hippocampus and neocortex underlying sleep-related memory consolidation (Siapas and Wilson, 1998; Sirota *et al.*, 2003; Axmacher *et al.*, 2006).

Ripples, at the same time, are linked to epileptic processes. In epilepsy patients focal seizures can be preceded by a distinct increase in high-frequency activity (Fisher *et al.*, 1992; Traub *et al.*, 2001; Worrell *et al.*, 2004; Jirsch *et al.*, 2006) and interictal epileptic spikes are frequently associated with ripples (Bragin *et al.*, 1999a,b; Staba *et al.*, 2002; Ulbert *et al.*, 2004). On this background, it is a matter of ongoing debate to what extent regular ripples detected in epilepsy patients reflect epileptic or regular physiological activity.

Human ripples share many characteristics with those in rodents, although human ripples are of lower frequency (80–140 Hz). So far, human studies on ripples relied on recordings with microelectrodes of 40 μm in diameter attached to clinical macroelectrodes (Bragin *et al.*, 1999a,b, 2002a,b; Staba *et al.*, 2002, 2004). In the present study we used parahippocampal foramen ovale (FO) electrodes and found that ripples can be likewise recorded via these semi-invasive macroelectrodes. The specific aim of our study was to explore whether a temporal coupling exists, similar to that seen in rodents, between parahippocampal ripples, the Non-REM sleep-slow oscillations and sleep spindles in epilepsy patients undergoing EEG monitoring with combined FO and scalp electrodes. We expected that not only spindle but also ripple activity in our patients is decreased during the slow oscillation down-state, and increased during the up-state. Because ripples can be part of the epileptic process we additionally aimed at clarifying to what extent the temporal coupling of slow oscillations and ripple activity is affected by the epileptic pathology.

Material and Methods

Patients

The study included seven medically intractable epilepsy patients implanted with FO electrodes and monitored in the National Institute of Psychiatry and Neurology, Budapest, Hungary. EEG monitoring with combined scalp and FO electrodes revealed mesiotemporal seizure onset in five patients. In two patients (patient #1 and #2) seizures originated from outside the mesiotemporal structures. Patient characteristics are summarized in Table 1. Patients gave written informed consent to the presurgical evaluation procedure including implantation of FO electrodes, long-term monitoring and using their EEG recordings for scientific analysis.

EEG recording

EEG monitoring with combined scalp and FO electrodes was carried out using the Brain Quick System98 (Micromed, Mogliano Veneto, Italy). Recording with FO electrodes is a semi-invasive technique allowing parahippocampal electrocorticography without opening the skull (Wieser *et al.*, 1985). FO electrodes are stainless steel wires with 0.65 mm diameter and four contacts of 2 mm length each. The four contacts have each a surface area of 4.1 mm² and are separated from each other by 5 mm (between contact centres). Impedance of the electrodes is 10 kOhm. The electrodes were introduced bilaterally through the foramen ovale such that the recording areas were placed in the subarachnoid space of the cisterna ambiens beneath the parahippocampal gyrus. The exact location of the FO electrodes was confirmed by X-ray scan (Fig. 1A and B) and in some cases by MRI (Fig. 1C and D). Additionally, electrodes were placed for recordings of the EEG from the scalp (according to the International 10–20 System), electrooculogram (EOG), electromyogram (EMG) and electrocardiogram (ECG). EEG signals were recorded to a vertex reference. Signals from all channels were filtered between 0.3 and 150 Hz, amplified and digitized with 22-bit resolution. Sampling rate was 512 Hz in all patients.

Determination of slow oscillations, spindle and ripple activity

EEG analysis was carried out using Spike2 software (Cambridge Electronic Design, Cambridge, UK). FO signals were analysed in bipolar montage, resulting in three bipolar channels bilaterally. Signals from scalp electrodes were re-referenced to contralateral mastoid electrodes, digitally filtered with a low-pass finite impulse

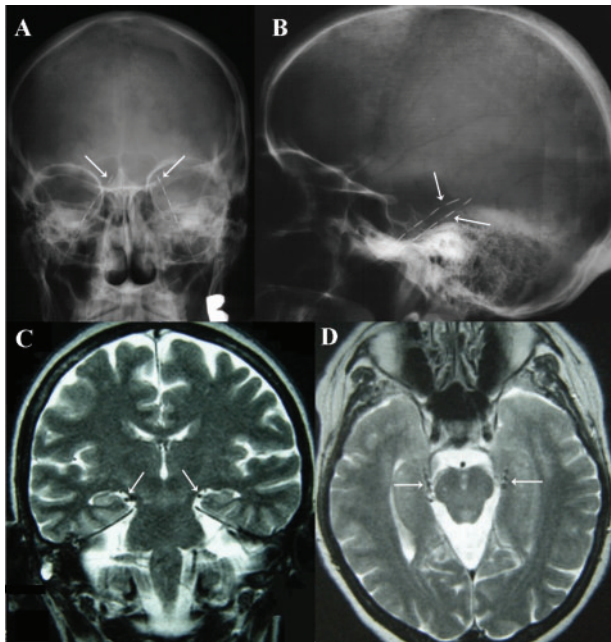


Fig. 1 X-ray views (**A**: coronal view, **B**: sagittal view) and MRI scans (**C**: coronal section, **D**: horizontal section) of the skull showing location of FO electrodes (arrows).

response (FIR) filter of 30 Hz (-3 dB at 32 Hz) and downsampled to 256 Hz. Artefacts in the EEG recordings were excluded by visual screening. In course of the clinical EEG monitoring period, several whole-night sleep recordings were stored for each patient. Out of these recordings, the night corresponding to the longest sleep period without epileptic seizures was selected for the present analysis.

First, sleep stages (Non-REM sleep 1, 2, 3, 4 and REM sleep), awake time and movement artefacts were scored for 30-s intervals (Rechtschaffen and Kales, 1968). Slow wave sleep (SWS) was defined by Non-REM stages 3 and 4. Then, within Non-REM sleep stages 2–4, the largest negative half-waves of the slow oscillation were detected in scalp EEG channels F3 and F4 by means of an automatic algorithm. Positions F3 and F4 were chosen because slow oscillations predominantly originate from prefrontal cortex (Massimini *et al.*, 2004) and, for reasons of consistency, these sites were used also in the other EEG analyses. EEG was first digitally filtered with a low-pass FIR filter of 3.1 Hz (-3 dB at 4 Hz). In the filtered trace negative half-waves were marked if the following criteria were fulfilled: (1) a negative and subsequent positive zero crossings separated by 0.3–1 s, (2) a negative peak between the two zero crossings of at least -20 μ V and (3) a negative-to-positive peak-to-peak amplitude of more than 30 μ V (Möller *et al.*, 2002). The latter two criteria were adjusted according to the patients' individual half-wave characteristics and to detect at least 1000 slow oscillation events during the whole sleep period. Detected events were marked by their peak negativity. Mean number (\pm SEM) of detected negative half-waves and amplitude criteria used are summarized in Table 2. To examine waveform characteristics of slow oscillations, the EEG signals (0.3–30 Hz) were averaged for intervals of ± 1 s around the peaks of all detected negative half-waves.

Table 2 Mean number \pm SEM of detected events during Non-REM sleep and mean thresholds \pm SEM used for detection

	Mean number	Mean threshold (μ V)
Slow oscillation negative half-waves	1675 \pm 242	-35 ± 1.1
Sleep spindles	788 \pm 112	7.4 \pm 0.6
Ripples	1934 \pm 357	3.3 \pm 0.7

Spindle and ripple activity was evaluated regarding two measures, i.e. the root mean square (RMS) amplitude within frequency bins of interest and the number of discrete events. To obtain the spindle activity signal the scalp EEG (0.3–30 Hz) in F3 and F4 was band-pass FIR filtered between 11 and 16 Hz (-3 dB at 10.5 and 16.5 Hz). Then spindle RMS amplitude was calculated at every sampling point using a 0.1-s window and the resulting RMS signal was smoothed with a moving average of 0.1 s. To examine the temporal dynamics between spindle activity and slow oscillations the spindle RMS signal was also averaged for intervals of ± 1 s around the peaks of all detected negative half-waves. For detection of discrete spindle events a thresholding procedure was applied to the band-pass filtered signal with an individually defined amplitude criterion. This criterion was determined based on visual inspection of recordings and identification of discrete sample spindles. A spindle was classified if consecutive peaks above the amplitude threshold occurred that were <90 ms apart and extended over more than 0.5 s. Detected spindles were marked by their peak in RMS activity, and again visually inspected to remove events contaminated by artefacts.

To obtain the ripple signal a band-pass FIR filter of 80–140 Hz (-3 dB at 78 and 142 Hz) was applied to all FO channels. The filter window was chosen based on previous studies in patients with temporal lobe epilepsy (Bragin *et al.*, 1999a,b; Staba *et al.*, 2002, 2004) which revealed hippocampal ripple frequencies between 80 and 150 Hz peaking at about 90 Hz. The ripple RMS amplitude was then calculated using a 0.02-s window. To detect discrete ripple events a thresholding procedure was applied to the band-pass filtered signal with an individually defined amplitude criterion. This criterion was determined separately for each channel (and patient) based on visual inspection of entire recordings and identification of discrete samples of ripple events (Fig. 2A). A ripple was detected if two succeeding peaks above the amplitude criterion occurred within <13.3 ms and if this held for at least three peaks in a row. Detected ripples were marked by their peak in RMS activity. A final visual inspection served to remove events that were contaminated by movement and other artefacts. To further validate our algorithm and to exclude that the detected ripple events reflected mainly filter artefacts caused by the sharp transients of epileptic spikes, we inspected in all patients samples of the unfiltered FO recording for intervals of detected ripple events. This inspection revealed that of the ripple events detected by the algorithm on average (i) $\sim 89\%$ showed a visually detectable ripple together with a spike or sharp wave (SPW), (ii) $\sim 9\%$ showed a visually detectable ripple without a spike or SPW, (iii) $<1\%$ showed only a spike but no clear ripple and (iv) $<1\%$ showed no apparent spike or ripple. On the other hand,

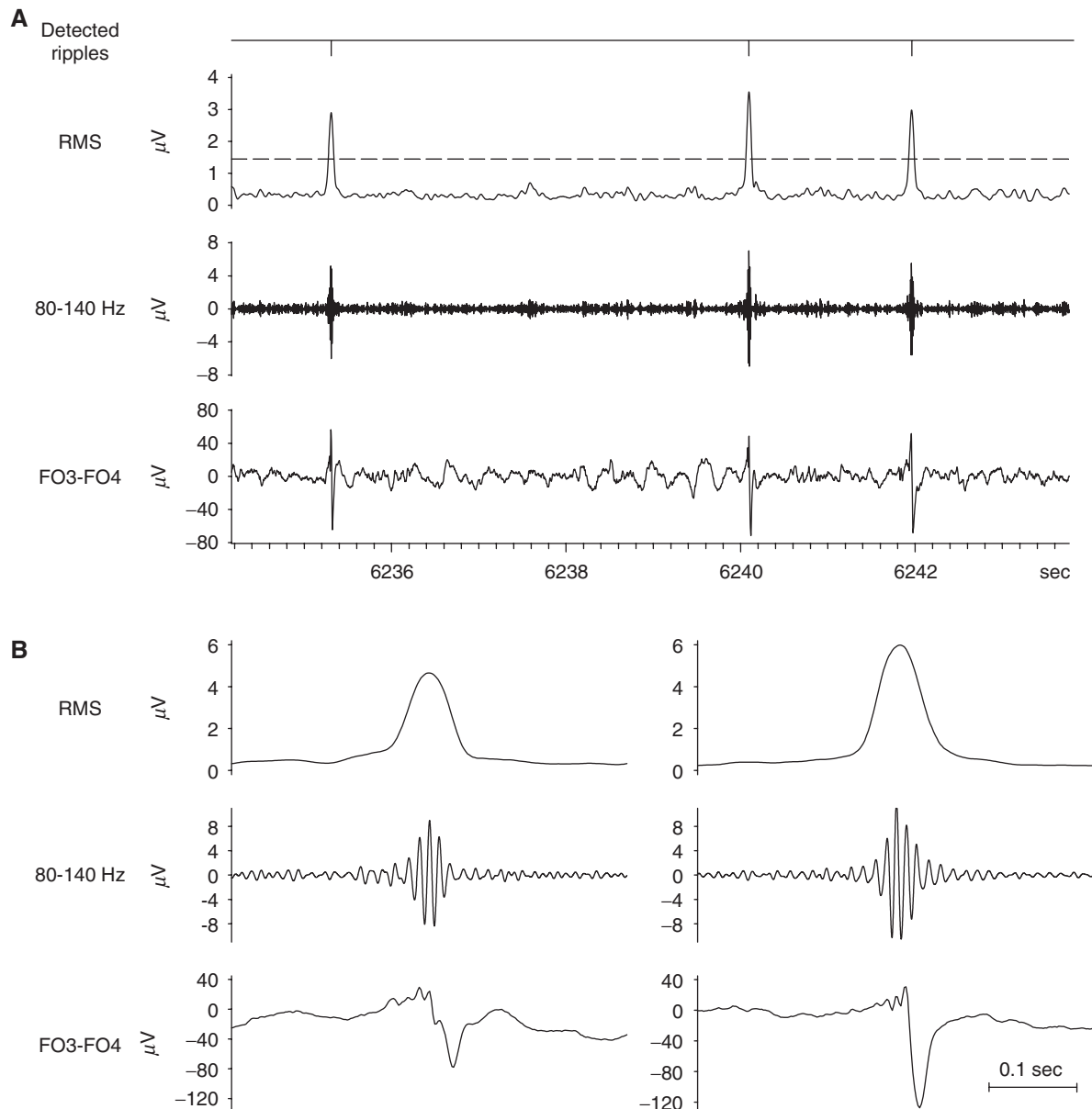


Fig. 2 (A) Detection of ripples. From bottom to top: unfiltered FO EEG trace containing three interictal epileptic spikes, trace band-pass filtered between 80 and 140 Hz, ripple root mean square (RMS) signal, event channel indicating detected ripples marked by their RMS peak. Horizontal dashed line illustrates the individually defined threshold used for ripple detection. (B) Examples of two individual ripples at a higher time resolution.

often spikes were seen in the unfiltered recordings where no ripple was detected. Mean (\pm SEM) numbers of spindles and ripples detected in individual recordings are summarized in Table 2.

To confirm an association between spike-like events or SPWs and ripples and to characterize ripple-associated slower waveforms, the unfiltered FO recordings were averaged time-locked to the peaks of detected ripples. To examine spectral properties of ripples, gated power spectra were calculated and averaged for a ± 0.125 -s interval around the peak of detected ripples (resulting in a 4 Hz resolution of the spectrum). To examine the occurrence of ripples in the different sleep and wake states, relative ripple density was calculated as the number of ripples per time spent

in these states divided by the individual average ripple density across all four states.

Statistical analyses

For investigating time relations between slow oscillations and ripple activity two types of analyses were carried out. First, the ripple RMS signal was averaged around the peaks of all detected negative half-waves using a ± 1 -s time window. In the second analysis, an event correlation histogram was calculated for the number of detected ripples with reference to the negative half-wave peak times in a 2-s window with an offset of 1 s and a bin

size of 16 ms. Histogram values were converted from counts to rate (events/s) in order to make values independent of bin size and total time of measurement (Abeles, 1982). So, the histogram represents the ripple rate at a given time before and after the peak of a slow oscillation negative half-wave. To examine time relations between ripple activity and spindles, also spindle triggered averages of ripple RMS and ripple rates were calculated using the spindle peak as reference. Waveform averages and event correlation histograms were always calculated for ipsilateral scalp and FO channels.

To statistically evaluate changes in RMS ripple activity associated with slow oscillations and spindles, the individual half-wave (or spindle) triggered averages were subjected first to *z*-transformation, because the means of the individual RMS time series showed considerable variability across patients. The transformation results in a mean of zero and a standard deviation of 1 for each patient's time series leaving the slow oscillation associated dynamics unaffected. After *z*-transformation the respective grand mean value was added to each data point to express values within the original range. Statistical comparisons relied on *t*-tests comparing average spindle and ripple RMS amplitudes and average numbers of detected ripple rates during defined intervals of the slow oscillation (or spindle) and the baseline period which was defined by the first 200 ms of the ± 1 -s window (Mölle *et al.*, 2002). Inspection of the individual slow oscillation averages confirmed that the potential level during this 200-ms interval was close to zero, thus representing a most 'neutral' point of reference in relation to the up and down-state of the slow oscillation. The slow oscillation averages showed a positive half-wave that followed the negative half-wave and peaked at about 0.5 s after the negative peak. Accordingly, we compared RMS activity and ripple rate during the negative slow oscillation half-waves (i.e. during a ± 0.1 -s interval around the negative peak) not only with those during the 200-ms baseline interval but also with those during the positive half-waves (i.e. during the interval 0.4–0.6 s following the negative half wave peak). Since results for the three bipolar FO channels within the same hemisphere were nearly identical, for statistical analysis only the two anterior FO bipolar channels (FO3-4 and FO7-8) were used, resulting in a total of 14 data sets (7 patients, 2 hemispheres). In one patient (patient #7) the posterior channels (FO1-2, FO5-6) were used, because of strong artefacts in the other channels. One other patient (patient #5) exhibited discrete ripples only unilaterally (left) and her data from the other hemisphere were not included in the analysis of discrete ripples.

Whereas the description of statistical results focuses on pairwise *t*-tests, additional analyses of variance (ANOVA) were performed which, aside from confirming the grouping effect of slow oscillations on ripple and spindle RMS, served to examine the dependency of this effect on the pathophysiology of epilepsy. Accordingly, these analyses included the repeated measures factor 'Phase' (representing the respective 200-ms intervals defining the baseline, negative half-wave and positive half-wave of the slow oscillation) and the group factors 'Site' (seizure inducing or not inducing) and 'Structural alteration'. The latter factor indicated the presence or absence of structural alterations in MRI scans of the temporal lobe. Of the three patients (#1, #2, #3) forming the group without temporal structural alterations, two had extratemporal epilepsy and one had temporal seizures for only 2 years. Structural alterations in the four patients of the other

group (#4, #5, #6, #7) were typical for temporal lobe epilepsy and these patients also had longer epilepsy durations (Table 1).

Results

In the FO recordings filtered between 80 and 140 Hz large amplitude sinusoid waves appeared as discrete events clearly distinguishable from background activity. We regarded these events as ripples since based on waveform characteristics they closely resembled those detected with microelectrodes in epilepsy patients in previous studies. Visual inspection of unfiltered recordings revealed a clear tendency (~89%) of these ripples to co-occur with spikes or SPW-like waves (Fig. 2). This was confirmed by calculating ripple-triggered waveform averages resulting in a waveform characteristic of an interictal epileptic spike (Fig. 3A). Although ripples often co-occurred in neighbouring channels (for a representative example see Fig. 1 of the Supplementary material), ripple-triggered averages on the same side differed to some extent between neighbouring channels. Typically in the averages the spike component was phase-inverted between anterior and middle bipolar FO channels. Similarities included that the ripple component was associated with an early component of the spike in each of the three channels on the same side. If present, ripples were observed in each bipolar FO channel although in most cases numbers of detected ripples decreased from anterior to posterior channels. Also, ripples occurred on both sides, except in one patient (#5) exhibiting ripples on the left side only. Notably, in this patient interictal epileptic spikes also appeared only on this side. Gated power spectra calculated for the unfiltered FO recording for a ± 0.125 -s interval around the peak of all detected ripples revealed a distinct peak around 84 ± 2.2 Hz (mean \pm SEM) in 10 of the 13 FO recordings (see Fig. 3B for a representative example). In the remaining three cases, no clear spectral peak was present in this frequency range.

Ripple density

Overall, ripple density was distinctly higher during Non-REM than REM sleep ($P < 0.001$). Differentiating Non-REM sleep stages revealed that the increase in ripple density during Non-REM sleep stage 2 ($P < 0.001$) and SWS ($P < 0.05$) were both significant in comparison to REM sleep, but only significant for sleep stage 2 and not for SWS when compared with wakefulness ($P < 0.01$ and $P = 0.49$, respectively, Fig. 4).

Spindle and ripple activity during slow oscillations

Visual inspection revealed that slow oscillation average waveforms in all patients were well comparable to those seen in healthy subjects (Mölle *et al.*, 2002; Massimini *et al.*, 2004) (Fig. 5A). The shape of spindle RMS averages time-locked to negative half-waves were also similar to that

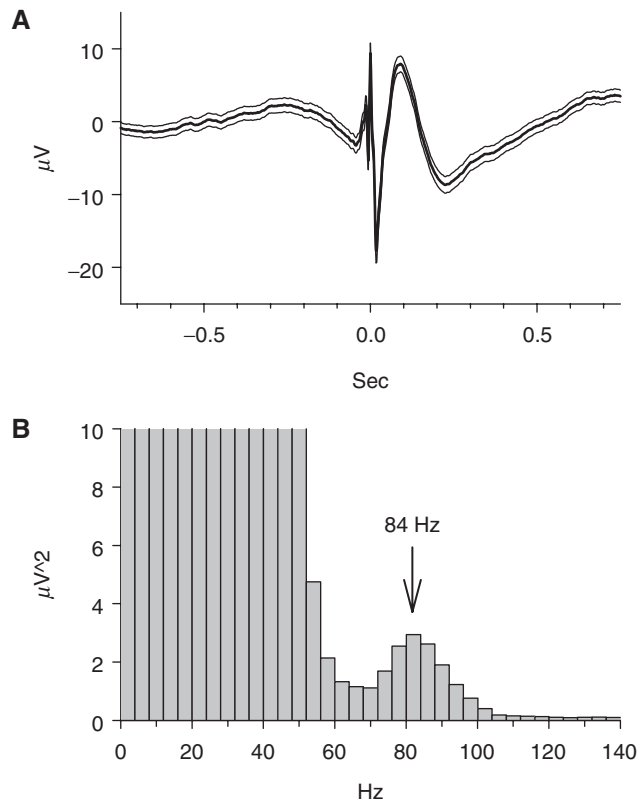


Fig. 3 (A) Unfiltered FO recordings averaged to all detected ripples in patient #4 (right FO, $n = 907$). Mean (thick line) values \pm SEM (thin lines) are indicated. A representative waveform is depicted. Average waveforms show some variability across neighbouring channels (see Supplementary material). Note, averaging results in a waveform typical also for an interictal epileptic spike. (B) Gated average power spectrum calculated (after ripple identification) on the unfiltered FO signal in a ± 0.125 -s interval around the peak of detected ripples in the same patient #4 (right FO). Note, the relatively low peak frequency is partly due to the limited bandwidth of our recordings, but likely reflects also a general tendency of human oscillations to be slower than in rats. The relatively low power at ripple frequency results from the use of unfiltered recordings.

seen in healthy controls (Möller *et al.*, 2002) in all but one patient (patient #7). Thus, spindle activity was suppressed around the negative peak of the slow oscillation followed by a rebound-like increase during subsequent slow oscillation positivity. (Refer to Fig. 2 of Supplementary material for representative original recordings illustrating the temporal relationship between slow oscillation negative half-waves and sleep spindle, as well as ripple activity.) In patient #7 the triggered spindle RMS signal curve considerably deviated from this pattern bilaterally (Fig. 5B). Nevertheless, statistical analyses over all cases as well as analyses excluding patient #7 confirmed a distinct decrease in spindle RMS in the interval 200 ms around the peak of the negative half-wave in comparison with the 200-ms interval of the succeeding positive half-wave and also in comparison with spindle activity

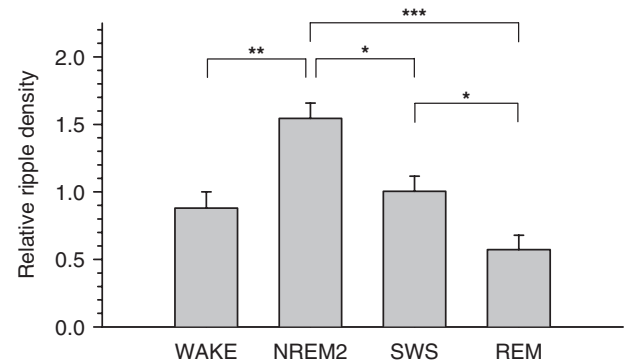


Fig. 4 Ripple density in the different sleep stages (Non-REM stage 2 and SWS, i.e. Non-REM stages 3 and 4) and during wakefulness. Asterisks indicate significant differences for pairwise comparisons (** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$). Relative ripple density is expressed as the number of ripples per time spent in a sleep stage divided by the individual average ripple density across all four stages. (Since slow oscillations as well as spindles can be reliably identified only during SWS and sleep stage 2, corresponding distributions are not shown.)

during baseline. RMS spindle activity (without patient #7) averaged during the negative half-wave $2.83 \pm 0.22 \mu\text{V}$, during the positive half-wave $4.97 \pm 0.21 \mu\text{V}$ ($P < 0.001$) and during baseline ($3.88 \pm 0.19 \mu\text{V}$, $P < 0.01$). Except in patient #7, temporal coupling of spindle activity to the slow oscillation did not appear to be essentially modulated by disease characteristics like the seizure inducing site or the presence of structural temporal lobe alterations in MRI scans ($P > 0.2$, for respective ANOVA interactions).

Averaging ripple RMS time-locked to the negative half-wave peak revealed a clear temporal relationship also between ripple activity and the slow oscillation. In an analysis across all patients, RMS ripple activity was distinctly reduced during the negative half-wave, i.e. in the 200-ms interval around the peak of the negative slow oscillation half-wave, in comparison with both the 200-ms baseline (-0.17 ± 0.21 versus $1.22 \pm 0.22 \mu\text{V}$, $P < 0.01$) and the 200-ms positive half-wave interval ($1.23 \pm 0.16 \mu\text{V}$, $P < 0.001$). Although present across all patients, this pattern was clearly more pronounced in the group of patients without structural temporal lobe alterations than in those displaying such alterations in the MRI ($P < 0.05$, for phase \times structural alteration). The seizure inducing site had no effect ($P > 0.2$). All patients without structural temporal alterations showed a distinct decrease in ripple RMS activity around the negative half-wave peak in recordings from both parahippocampi (-0.78 ± 0.14 versus $1.48 \pm 0.20 \mu\text{V}$ during baseline, $P < 0.001$; versus $1.26 \pm 0.23 \mu\text{V}$ during the positive half-wave interval, $P < 0.01$; Fig. 5C, right panel). In the patients showing structural temporal alterations the decrease in ripple RMS during slow oscillation negativity was less consistent or even absent (patient #7) and failed to reach significance if compared to the baseline

(0.28 ± 0.25 versus $1.03 \pm 0.34 \mu\text{V}$, $P > 0.2$) or positive half-wave ($1.20 \pm 0.22 \mu\text{V}$, $P < 0.06$).

Inspection of event correlation histograms also revealed a decrease in discrete ripples around the negative peak of the slow oscillation which again was clearly visible only for the patients without temporal structural alterations in the MRI scan. Across all patients the decrease in ripple rate during the negative half-wave reached significance for the comparison against the baseline (0.114 ± 0.024 versus 0.139 ± 0.023 Hz, $P < 0.01$) but failed to do so for the comparison against the positive half-wave (0.133 ± 0.022 Hz, $P = 0.1$). However, in the subgroup of patients without temporal structural alterations, ripple rate was consistently decreased during slow oscillation negativity in comparison with both the baseline (0.080 ± 0.016 versus 0.117 ± 0.021 Hz, $P < 0.01$) and positive half-wave interval (0.109 ± 0.018 Hz, $P < 0.01$). In contrast, no such pattern was present in the patients with structural temporal alterations (0.144 ± 0.040 versus 0.157 ± 0.039 Hz during baseline, $P > 0.4$, and 0.153 ± 0.038 Hz during positive half-wave, $P > 0.7$).

Ripple activity during spindles

Ripple RMS averaged time-locked to spindles revealed a remarkably robust temporal relationship that was evident in 13 of the 14 parahippocampal recordings. Compared with the 1-s interval following the spindle peak, ripple RMS was clearly increased during the 1-s interval preceding the spindle peak (1.77 ± 0.07 versus $0.72 \pm 0.07 \mu\text{V}$, $P < 0.001$; Fig. 5E). The increase in ripple RMS reaching a maximum between -0.35 and -0.15 s before the spindle peak was also significant if compared to the 200-ms baseline in the subgroup of patients without distinct temporal structural alterations (2.34 ± 0.17 versus $1.50 \pm 0.17 \mu\text{V}$, $P < 0.05$), but failed to do so across all patients (1.98 ± 0.14 versus $1.54 \pm 0.13 \mu\text{V}$, $P < 0.08$). In the event correlation histograms, the relationship between ripples and spindles was less clear, but an increased ripple frequency during the 1-s interval before as compared to the 1-s interval following spindle peaks was still present in 9 of the 13 parahippocampi recorded. Analyses across all recordings confirmed significance of this relationship (0.171 ± 0.029 versus 0.145 ± 0.023 Hz, $P < 0.05$; Fig. 5F).

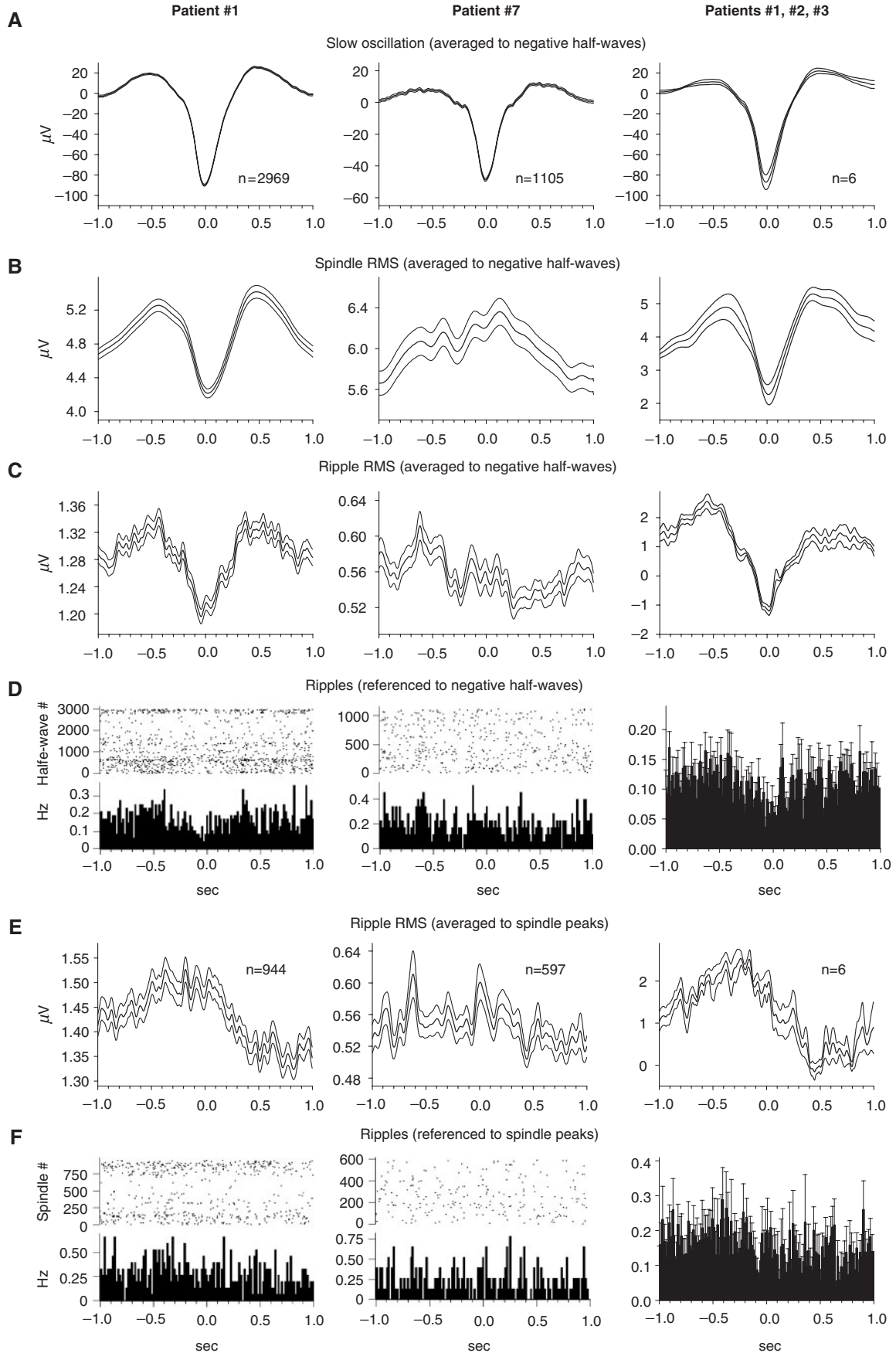
Discussion

The present results provide first evidence for a temporal coupling between parahippocampal ripples, sleep spindles and slow oscillations in humans. Confirming our hypothesis the neocortical slow oscillation induced a grouping effect such that spindle and ripple activity were distinctly decreased during the negative slow oscillation half-wave, i.e. down-state, and increased during the subsequent positive up-state. Whereas a coupling between slow oscillations and spindle activity was a quite consistent

finding in our patients, the synchronization of ripples to the slow oscillation was clearly more pronounced in the patients whose MRI scans did not indicate severe structural alterations in mesiotemporal regions.

In healthy humans the hyperpolarizing down-state of the slow oscillation has been shown to be associated with decreased spindle activity, whereas the subsequent depolarizing, surface-positive state is associated with a rebound-like increase in spindle activity (Mölle *et al.*, 2002) possibly reflecting post-inhibitory rebound of discharge bursts of thalamocortical neurons (Contreras and Steriade, 1995). In rats and mice the grouping effect of the cortical slow oscillation is not limited to activity within neocortical and thalamocortical circuitry but pertains to hippocampal ripple activity as well, such that ripple activity becomes likewise suppressed during the slow oscillation down-state and increases in parallel with the up-state (Sirota *et al.*, 2003; Battaglia *et al.*, 2004; Mölle *et al.*, 2006; Isomura *et al.*, 2006). Also, a distinct temporal association exists in rodents between the occurrence of sleep spindles and hippocampal sharp wave–ripple complexes (Siapas and Wilson, 1998; Sirota *et al.*, 2003). Notably, this coordinate interaction between hippocampal and thalamocortical networks has been suggested to provide a temporal framework for information transfer underlying the consolidation of hippocampus-dependent memories during sleep (Buzsáki, 1989, 1998; Sirota *et al.*, 2003; Gais and Born, 2004; Born *et al.*, 2006). A central assumption of these concepts is that sleep-associated memory consolidation relies on a replay of recently encoded memories which takes place in hippocampal cells during slow-wave sleep in temporal association with sharp wave–ripples (Kudrimoti *et al.*, 1999; Nádasdy *et al.*, 1999; Rasch *et al.*, 2007; Ji and Wilson, 2007). The neuronal replay during sharp wave–ripples is thought to stimulate the transfer of the newly encoded memories to neocortical networks for long-term storage. In this process, coactivation of spatially

Fig. 5 Examples of average waveforms and event correlation functions in patient #1 with extratemporal epilepsy and negative MRI scans (left panels) and in patient #7 with severe temporal structural alterations (middle). Right panels show average waveforms and event correlation histograms across both hemispheres in the three patients (patients #1, #2 and #3) without temporal lobe structural alterations. Data in panels **A–D** are time-locked to the peak of negative slow oscillation half-waves, data in panels **E** and **F** are time-locked to the peak of spindles. From top to bottom: **A**, time-locked average (\pm SEM) of slow oscillations; **B**, of spindle root mean square (RMS) signal and **C**, of ripple RMS signal. (RMS signals were z-transformed and the grand mean value was added before analysis.) Note abnormal shape of the triggered spindle RMS curve in patient #7. **D**, event correlation function for the number of detected ripples time-locked to negative slow oscillation half-wave peaks. **E**, averaged (\pm SEM) ripple RMS and **F**, event correlation function for number of detected ripples both time-locked to spindle peaks. Event correlation functions of the two patients (**D** and **F**, left and middle) are displayed together with corresponding raster plots and values were converted from counts to rate (in Hz).



distinct networks within the hippocampus, thalamus and neocortex might be brought about by the slow oscillations that are generated in neocortical circuitry and exert a synchronizing influence on both thalamic spindle and hippocampal ripple activity, thereby facilitating hippocampo-neocortical information transfer. There is also evidence that sleep spindles provide ideal conditions for long-term potentiation through eliciting massive Ca^{2+} entry in spindling pyramidal cells (Sejnowski and Destexhe, 2000; Rosanova and Ulrich, 2005). Data from human behavioural studies have likewise supported an involvement of both slow oscillations (Huber *et al.*, 2004; Mölle *et al.*, 2004; Marshall *et al.*, 2006) and spindles (Gais *et al.*, 2002; Schabus *et al.*, 2004; Clemens *et al.*, 2005a, 2006) in sleep-related memory consolidation.

Our data show that also in humans ripple activity from parahippocampal recordings is synchronized to neocortical slow oscillations such that ripple activity is distinctly decreased during the negative slow-oscillation half-wave, i.e. down-state, and increased during the subsequent positive up-state. The finding suggests that slow oscillations play a coordinating role in the hippocampo-neocortical dialogue underlying memory consolidation during sleep in humans as well. It is to be noted, however, that the temporal association between slow oscillations and ripple activity was revealed only in the three patients without distinct structural damage to mesiotemporal regions, whereas it was less consistent or absent in those patients exhibiting such structural alterations. Patients with temporal lobe epilepsy have been found to display impairments of sleep-associated memory consolidation (Clemens *et al.*, 2003). Hence, a tempting hypothesis is that the patients with obvious mesiotemporal lesions and lacking synchronization between slow oscillations and ripple activity show also a more severe impairment of memory consolidation during sleep, as compared with the patients showing synchrony between ripples and slow oscillations.

Slow oscillations of neocortical origin probably reach the hippocampus via the temporo-ammonic pathway (e.g. Wolansky *et al.*, 2006), and it may be lesions to this pathway that abolished the temporal link between slow oscillations and ripples in the patients with clearly identifiable temporal structural alterations. Also, patients with such structural alterations typically show increased EEG rigidity that might add to the difference in slow oscillation-related changes in ripple activity between the two patient subgroups (Wang and Wieser, 1994). Similarly, increased spiking stability and a diminished sleep-related spike modulation was found in the patients' primary epileptogenic region (Rossi *et al.*, 1984) and in patients with long-standing epilepsy (Clemens *et al.*, 2005b).

Our data show a clear temporal association also between ripple and spindle activity which confirms and extends previous observations in rats (Siapas and Wilson, 1998; Mölle *et al.*, 2006). Ripple activity was increased shortly before spindle peaks and decreased distinctly thereafter.

This temporal association which again was most robust in the subgroups of patients without structural temporal lobe alteration, might reflect a common driving influence of the slow oscillation on both the thalamic generation of spindles and the hippocampal generation of ripples, but it could also reflect a direct interaction between spindles and ripples, although the pathways and mediating mechanisms remain obscure. That ripple activity was significantly higher before than after spindle peaks suggests a driving influence of ripples on spindles, and would not be compatible with the inverse relationship. In rats, corresponding evidence that during ripple-spindle events hippocampal firing tends to precede the onset of spindles was revealed by Siapas and Wilson (1998). Those authors suggested that effective hippocampo-neocortical information transfer during sleep is bound to conditions whereby hippocampal excitation feeds into subsequent spindle cycles. Of note, this view does not rule out that the exact timing of ripple-spindle events is subject to a synchronizing influence of the slow oscillation.

Ripples identified here with FO electrodes from parahippocampal sites were similar in shape to those observed in earlier studies in patients using intrahippocampal microelectrodes (e.g. Staba *et al.*, 2002, 2004). We found ripples to be closely associated with activity characteristic of interictal epileptic spikes which corroborates findings of a previous study (Ulbert *et al.*, 2004), although in other studies the coincidence of ripples with spikes was less clear (Bragin *et al.*, 1999a,b; Staba *et al.*, 2002). Those latter studies, additionally identified fast ripples (>200 Hz) in epilepsy patients (Bragin *et al.*, 1999a,b, 2002a,b; Staba *et al.*, 2002) which due to a lower sampling rate could not be addressed here. It might be argued that with the algorithm applied here, which was similar to that used in previous patient studies (e.g. Bragin *et al.*, 1999a,b), we detected only a specific subpopulation of ripples. Considering our strict criteria for selecting the largest and most regular ripples, this subpopulation of ripples might in fact encompass specifically those events that are most similar to pathological interictal spikes with superimposed fast oscillations. This would explain that our data suggest a high degree of similarity between physiological sharp wave-ripple events and interictal spikes accompanied by ripple-like oscillations, with both phenomena just representing different points on the same continuum. Based on the selection procedures applied to epileptic brains we basically cannot safely dissociate pathological from normal fast oscillatory events. However, smaller and less clear-cut ripples that were not detected by the algorithm contributed to the ripple RMS measure (although this measure to some extent catches also unspecific background activity). Notably, for RMS ripple activity the modulation by the slow oscillation as well as the link to spindles was even stronger than for detected ripple events. Assuming that RMS ripple activity is less confounded by epileptogenic activity,

this pattern suggests an even stronger temporal coupling to the slow oscillation of normal ripple activity.

Interictal epileptic spikes have a complex relation to the epileptogenic process and seizures (De Curtis and Avanzini, 2001). Typically spikes are not limited to the primary epileptogenic region but can arise from larger areas that were recruited in previous seizures (Janszky *et al.*, 2001; Janszky and Ebner, 2002). Spike activity from these areas is thought to reflect irritation of the tissue (Rosenow and Lüders, 2001). Given the strong association between ripples and spikes, spike-associated ripples might reflect irritative properties of these tissues as well. However, without necessarily contradicting this, spike-associated ripples could likewise originate from normal physiological ripples. This view is supported by findings indicating that ripple characteristics are comparable in normal and in epileptic (kindled) rats, and only the amplitude of the associated sharp waves is higher in epileptic rats (Bragin *et al.*, 1999b). Both sharp waves (Buzsáki, 1986) and interictal epileptic spikes (Avoli, 2001) originate from the hippocampal CA3 region. Also the epileptic process is well-known to be characterized by a general tendency of hypersynchronisation and exaggeration of normal oscillations, as seen for example in the epileptic facilitation of sleep spindles (Steriade *et al.*, 1994; Clemens and Ménes, 2000). On this background the spike–ripple complex might well represent an epileptic transformation of the normal sharp wave–ripple complex.

In sum the present data indicate in epileptic humans a temporal coupling not only of spindles but also of parahippocampally recorded ripple activity to the neocortical slow oscillation. However, the grouping effect was hampered in patients showing structural alterations in MRI scans of the mesiotemporal lobe, with this impairment possibly contributing to deficits in sleep-related memory consolidation and long-term memory that have been observed in temporal lobe epilepsy patients (Blake *et al.*, 2000; Clemens *et al.*, 2003).

Supplementary material

Supplementary material is available at *Brain* online.

Acknowledgements

This work was supported by grants from the BIAL Foundation (Portugal, No. 168/04) and the Deutsche Forschungsgemeinschaft (SFB 654, “Plasticity and Sleep”).

References

- Ables M. Quantification, smoothing, and confidence limits for single-units' histograms. *J Neurosci Methods* 1982; 5: 317–25.
- Achermann P, Borbély AA. Low-frequency (<1 Hz) oscillations in the human sleep electroencephalogram. *Neuroscience* 1997; 81: 213–22.
- Avoli M. Do interictal discharges promote or control seizures? Experimental evidence from an in vitro model of epileptiform discharge. *Epilepsia* 2001; 42 (Suppl 3): 2–4.
- Axmacher N, Mormann F, Fernandez G, Elger CE, Fell J. Memory formation by neuronal synchronization. *Brain Res Rev* 2006; 52: 170–82.
- Battaglia FP, Sutherland GR, McNaughton BL. Hippocampal sharp wave bursts coincide with neocortical “up-state” transitions. *Learn Mem* 2004; 11: 697–704.
- Behrens CJ, van den Boom LP, de HL, Friedman A, Heinemann U. Induction of sharp wave-ripple complexes in vitro and reorganization of hippocampal networks. *Nat Neurosci* 2005; 8: 1560–7.
- Blake RV, Wroe SJ, Breen EK, McCarthy RA. Accelerated forgetting in patients with epilepsy: evidence for an impairment in memory consolidation. *Brain* 2000; 123 (Pt 3): 472–83.
- Born J, Rasch BH, Gais S. Sleep to remember. *Neuroscientist* 2006; 12: 410–24.
- Bragin A, Engel J, Jr, Wilson CL, Fried I, Buzsáki G. High-frequency oscillations in human brain. *Hippocampus* 1999a; 9: 137–42.
- Bragin A, Engel J, Jr, Wilson CL, Fried I, Mathern GW. Hippocampal and entorhinal cortex high-frequency oscillations (100–500 Hz) in human epileptic brain and in kainic acid–treated rats with chronic seizures. *Epilepsia* 1999b; 40: 127–37.
- Bragin A, Mody I, Wilson CL, Engel J, Jr. Local generation of fast ripples in epileptic brain. *J Neurosci* 2002a; 22: 2012–21.
- Bragin A, Wilson CL, Staba RJ, Reddick M, Fried I, Engel J, Jr. Interictal high-frequency oscillations (80–500 Hz) in the human epileptic brain: entorhinal cortex. *Ann Neurol* 2002b; 52: 407–15.
- Buzsáki G. Memory consolidation during sleep: a neurophysiological perspective. *J Sleep Res* 1998; 7: 17–23.
- Buzsáki G. Hippocampal sharp waves: their origin and significance. *Brain Res* 1986; 398: 242–52.
- Buzsáki G. Two-stage model of memory trace formation: a role for “noisy” brain states. *Neuroscience* 1989; 31: 551–70.
- Buzsáki G, Draguhn A. Neuronal oscillations in cortical networks. *Science* 2004; 304: 1926–9.
- Clemens B, Ménes A. Sleep spindle asymmetry in epileptic patients. *Clin Neurophysiol* 2000; 111: 2155–9.
- Clemens Z, Clemens B, Janszky J, Szücs A, Rásonyi G, Halász P. Memory consolidation during sleep in epilepsy patients. *Epilepsia* 2003; 44: 72.
- Clemens Z, Fabó D, Halász P. Overnight verbal memory retention correlates with the number of sleep spindles. *Neuroscience* 2005a; 132: 529–35.
- Clemens Z, Fabó D, Halász P. Twenty-four hours retention of visuospatial memory correlates with the number of parietal sleep spindles. *Neurosci Lett* 2006; 403: 52–6.
- Clemens Z, Janszky J, Clemens B, Szücs A, Halász P. Factors affecting spiking related to sleep and wake states in temporal lobe epilepsy (TLE). *Seizure* 2005b; 14: 52–7.
- Contreras D, Steriade M. Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. *J Neurosci* 1995; 15: 604–22.
- Csicsvári J, Hirase H, Czurkó A, Mamiya A, Buzsáki G. Fast network oscillations in the hippocampal CA1 region of the behaving rat. *J Neurosci* 1999; 19: RC20.
- De Curtis M, Avanzini G. Interictal spikes in focal epileptogenesis. *Prog Neurobiol* 2001; 63: 541–67.
- Destexhe A, Contreras D, Steriade M. Spatiotemporal analysis of local field potentials and unit discharges in cat cerebral cortex during natural wake and sleep states. *J Neurosci* 1999; 19: 4595–608.
- Fisher RS, Webber WR, Lesser RP, Arroyo S, Uematsu S. High-frequency EEG activity at the start of seizures. *J Clin Neurophysiol* 1992; 9: 441–8.
- Gais S, Born J. Declarative memory consolidation: mechanisms acting during human sleep. *Learn Mem* 2004; 11: 679–85.
- Gais S, Mölle M, Helms K, Born J. Learning-dependent increases in sleep spindle density. *J Neurosci* 2002; 22: 6830–4.
- Huber R, Ghilardi MF, Massimini M, Tononi G. Local sleep and learning. *Nature* 2004; 430: 78–81.
- Isomura Y, Sirota A, Ozen S, et al. Integration and segregation of activity in entorhinal-hippocampal subregions by neocortical slow oscillations. *Neuron* 2006; 52: 871–82.
- Janszky J, Ebner A. Interictal spikes: signs of a negative-feedback mechanism of epilepsy? *Epilepsia* 2002; 43: 665.

- Janszky J, Fogarasi A, Jokeit H, Schulz R, Hoppe M, Ebner A. Spatiotemporal relationship between seizure activity and interictal spikes in temporal lobe epilepsy. *Epilepsy Res* 2001; 47: 179–88.
- Ji D, Wilson MA. Coordinated memory replay in the visual cortex and hippocampus during sleep. *Nat Neurosci* 2007; 10: 100–7.
- Jirsch JD, Urrestarazu E, LeVan P, Olivier A, Dubeau F, Gotman J. High-frequency oscillations during human focal seizures. *Brain* 2006; 129: 1593–608.
- Kudrimoti HS, Barnes CA, McNaughton BL. Reactivation of hippocampal cell assemblies: effects of behavioral state, experience, and EEG dynamics. *J Neurosci* 1999; 19: 4090–101.
- Marshall L, Helgadottir H, Mölle M, Born J. Boosting slow oscillations during sleep potentiates memory. *Nature* 2006; 444: 610–3.
- Massimini M, Huber R, Ferrarelli F, Hill S, Tononi G. The sleep slow oscillation as a traveling wave. *J Neurosci* 2004; 24: 6862–70.
- Mölle M, Marshall L, Gais S, Born J. Learning increases human electroencephalographic coherence during subsequent slow sleep oscillations. *Proc Natl Acad Sci USA* 2004; 101: 13963–8.
- Mölle M, Marshall L, Gais S, Born J. Grouping of spindle activity during slow oscillations in human non-rapid eye movement sleep. *J Neurosci* 2002; 22: 10941–7.
- Mölle M, Yeshenko O, Marshall L, Sara SJ, Born J. Hippocampal sharp wave-ripples linked to slow oscillations in rat slow-wave sleep. *J Neurophysiol* 2006; 96: 62–70.
- Nádasdy Z, Hirase H, Czurkó A, Csicsvari J, Buzsáki G. Replay and time compression of recurring spike sequences in the hippocampus. *J Neurosci* 1999; 19: 9497–507.
- Rasch B, Büchel C, Gais S, Born J. Odor cues during slow-wave sleep prompt declarative memory consolidation. *Science* 2007; 315: 1426–9.
- Rechtschaffen A, Kales A. A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Los Angeles: Brain Information Service; 1968.
- Rosanov M, Ulrich D. Pattern-specific associative long-term potentiation induced by a sleep spindle-related spike train. *J Neurosci* 2005; 25: 9398–405.
- Rosenow F, Lüders H. Presurgical evaluation of epilepsy. *Brain* 2001; 124: 1683–700.
- Rossi GF, Colicchio G, Pola P. Interictal epileptic activity during sleep: a stereo-EEG study in patients with partial epilepsy. *Electroencephalogr Clin Neurophysiol* 1984; 58: 97–106.
- Schabus M, Gruber G, Parapatics S, et al. Sleep spindles and their significance for declarative memory consolidation. *Sleep* 2004; 27: 1479–85.
- Sejnowski TJ, Destexhe A. Why do we sleep? *Brain Res* 2000; 886: 208–23.
- Siapas AG, Wilson MA. Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. *Neuron* 1998; 21: 1123–8.
- Sirota A, Csicsvari J, Buhl D, Buzsáki G. Communication between neocortex and hippocampus during sleep in rodents. *Proc Natl Acad Sci USA* 2003; 100: 2065–9.
- Staba RJ, Wilson CL, Bragin A, Fried I, Engel J, Jr. Quantitative analysis of high-frequency oscillations (80–500 Hz) recorded in human epileptic hippocampus and entorhinal cortex. *J Neurophysiol* 2002; 88: 1743–52.
- Staba RJ, Wilson CL, Bragin A, Jhung D, Fried I, Engel J, Jr. High-frequency oscillations recorded in human medial temporal lobe during sleep. *Ann Neurol* 2004; 56: 108–15.
- Steriade M. Grouping of brain rhythms in corticothalamic systems. *Neuroscience* 2006; 137: 1087–106.
- Steriade M, Contreras D, Amzica F. Synchronized sleep oscillations and their paroxysmal developments. *Trends Neurosci* 1994; 17: 199–208.
- Steriade M, Contreras D, Curro DR, Nunez A. The slow (< 1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. *J Neurosci* 1993a; 13: 3284–99.
- Steriade M, Nunez A, Amzica F. A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *J Neurosci* 1993b; 13: 3252–65.
- Traub RD, Whittington MA, Buhl EH, et al. A possible role for gap junctions in generation of very fast EEG oscillations preceding the onset of, and perhaps initiating, seizures. *Epilepsia* 2001; 42: 153–70.
- Ulbert I, Heit G, Madsen J, Karmos G, Halgren E. Laminar origin of ripple oscillations (80–160 Hz) associated with interictal spikes in the human neocortex. *Human Brain Mapping Conference*; 2004. Ref Type: Conference Proceeding.
- Wang J, Wieser HG. Regional “rigidity” of background EEG activity in the epileptogenic zone. *Epilepsia* 1994; 35: 495–504.
- Wieser HG, Elger CE, Stodieck SR. The ‘foramen ovale electrode’: a new recording method for the preoperative evaluation of patients suffering from mesio-basal temporal lobe epilepsy. *Electroencephalogr Clin Neurophysiol* 1985; 61: 314–22.
- Wolansky T, Clement EA, Peters SR, Palczak MA, Dickson CT. Hippocampal slow oscillation: a novel EEG state and its coordination with ongoing neocortical activity. *J Neurosci* 2006; 26: 6213–29.
- Worrell GA, Parish L, Cranston SD, Jonas R, Baltuch G, Litt B. High-frequency oscillations and seizure generation in neocortical epilepsy. *Brain* 2004; 127: 1496–506.