Note

Sleep-dependent directional coupling between human neocortex and hippocampus

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A B S T R A C T
Complex interactions between neocortex and hippocampus are the neural basis of memory formation. Two-step theories of memory formation suggest that initial encoding of novel information depends on the induction of rapid plasticity within the hippocampus, and is followed by a second sleep-dependent step of memory consolidation. These theories predict information flow from the neocortex into the hippocampus during waking state and in the reverse direction during sleep. However, experimental evidence that interactions between hippocampus and neocortex have a predominant direction which reverses during sleep rely on cross-correlation analysis of data from animal experiments and yielded inconsistent results. Here, we investigated directional coupling in intracranial EEG data from human subjects using a phase-modeling approach which is well suited to reveal functional interdependencies in oscillatory data. In general, we observed that the anterior hippocampus predominantly drives nearby and remote brain regions. Surprisingly, however, the influence of neocortical regions on the hippocampus significantly increased during sleep as compared to waking state. These results question the standard model of hippocampal-neocortical interactions and suggest that sleep-dependent consolidation is accomplished by an active retrieval of hippocampal information by the neocortex.

1. Introduction

The direction of information flow between neocortex and hippocampus is an important and yet unresolved issue in memory research (Tononi et al., 2006). Two-step theories of memory formation suggest that, in order to build stable memory traces, a first step of encoding needs to be followed by a second step of memory consolidation (Marr, 1971; Buzsáki, 1989, 1998; McClelland et al., 1995; Squire and Alvarez, 1995; Hasselmo, 1999). Initial encoding, which occurs during exploratory stages of waking behaviour, likely depends on the transfer of information from sensory neocortical regions into the hippocampus. According to the standard model of memory consolidation, activity within the hippocampus is replayed during consecutive sleep periods and triggers the formation of stable memory traces in the neocortex (e.g., Maquet, 2001; Gais

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and Born, 2004; Wiltgen et al., 2004; Stickgold and Walker, 2005). Therefore, this theory suggests that the direction of hippocampal-neocortical interactions should reverse during the wake-sleep cycle: from the neocortex into the hippocampus during waking state and in the opposite direction during sleep.

This theory offers a seductive and intuitively convincing account on the role of hippocampal–neocortical interactions for memory formation. However, it has been questioned by several recent studies. While hippocampal ‘ripples’ bursts with a frequency of around 200 Hz (Buzsáki et al., 1992) and a repetition rate of around 2 per minute (Axmacher et al., 2008) are followed by neocortical sleep spindles on a broad time scale (Siapas and Wilson, 1998), analysis on a finer time scale revealed that ripples are actually triggered by neocortical slow oscillations (Sirotta et al., 2003; Isomura et al., 2006; Mölle et al., 2006). Based on these studies, Tononi et al. (2006) recently proposed an alternative scenario, where the information flow between neocortex and hippocampus is bidirectional during wakefulness and the neocortex predominantly influences the hippocampus during sleep.

The aim of the current study was to explore the directional coupling between neocortex, rhinal cortex, and hippocampus in the human brain during different sleep stages. We analyzed intracranial electroencephalogram (EEG) data from sleep and waking state in epilepsy patients undergoing presurgical investigation. In order to measure directionality, we used an approach (Rosenblum and Pikovsky, 2001), which quantifies the direction of coupling between the phases of two oscillatory (sub-)systems. For this purpose, the deterministic part of the underlying phase dynamics is modeled including functional dependencies between both phases. Main features of this method are robustness against noise and small influence of frequency mismatch (Rosenblum and Pikovsky, 2001; Osterhage et al., 2007). This technique has been successfully applied not only to model systems (e.g., Cimponeriu et al., 2003), but also to EEG data from epileptic patients (Osterhage et al., 2007), as well as in a magnetoencephalogram (MEG) motor control experiment (Gross et al., 2002). Taken together, this approach allows one to identify asymmetry of directional coupling more accurately than usually employed cross-correlation measures.

2. Methods

2.1. EEG recordings and sleep staging

We investigated all night sleep scalp and intracranial EEG recordings from nine patients with pharmacoresistant, unilateral medial temporal lobe epilepsy – for details see Table 1. The scalp EEG was recorded from positions C3, C4, and O1 (10–20 system). Furthermore, electro-ocular activity was acquired at the outer canthi of both eyes, and submental electromyographic activity was registered with electrodes fixed at the skin. All interelectrode impedances were below 5 kΩ. Intracranial EEG were recorded from bilateral depth electrodes each with 10 platinum contacts that had been stereotactically implanted along the longitudinal axes of both medial temporal lobes (MTL) for presurgical evaluation of the seizure onset zone. The scalp and intracranial EEGs were referenced to linked mastoids, bandpass-filtered [0.1 Hz (6 dB/octave)–70 Hz (12 dB/octave)], and simultaneously recorded with a sampling rate of 200 Hz using a 12 bit analog to digital converter. For each patient, the position of depth electrode contacts was identified by inspection of post implantation magnetic resonance imaging scans (sagittal, axial and coronal planes) (Van Roost et al., 1998). For further analysis, intracranial electrode contacts were selected based on anatomical criteria: according to the functional differentiation within the medial temporal lobe (Moser and Moser, 1998; Fernández et al., 1999), contacts that recorded electrical activity within the anterior (AH) and posterior hippocampus (PH) – anterior and posterior third – and the rhinal cortex (RC) covering the anterior parahippocampal gyrus were chosen. The RC not only represents the anatomical bottleneck between neocortex and hippocampus, but also seems to play an active role as ‘gatekeeper’ for the declarative memory system controlling the information transfer from the neocortex to the hippocampus (Fernández and Tendolkar, 2006). In other words, it filters incoming new information according to criteria like novelty or salience and selects only relevant information for further processing and long-term memory encoding. Additionally, we investigated EEG recordings from surface contacts C3/C4 as electrical activity of neocortical regions (NC), because these electrode positions were used in all patients to conform with Rechtschaffen/Kales criteria for sleep stages (Rechtschaffen and Kales, 1968). In each patient a unilateral seizure origin within one MTL (five patients – right side; four patients – left side) was diagnosed. Analyses rely on scalp and intracranial EEG data obtained from the non-affected hemisphere. The study has been approved by the local institutional ethics committee and was accomplished with informed consent from all patients.

The continuous EEG recordings were split into epochs of 20 sec duration. For each epoch, the sleep stage was classified by visual inspection of scalp EEG pursuant to the standard rules (Rechtschaffen and Kales, 1968) as sleep stages 1–4, rapid eye movement (REM) sleep, or waking state. Stage 3 and 4 were pooled together as slow wave sleep (SWS). EEG epochs were inspected twice for movement artefacts and epileptiform activity. If present, epochs were discarded. Hence, 66.4% of all EEG epochs were excluded from further analysis. An overview of total numbers of epochs is presented at the end of the following section. During waking state before sleep onset, the patients did not take part in explicitly controlled tasks but were engaged in non-demanding activities, like hearing music, watching TV, or reading.

2.2. Directional analysis

Recent studies applied bivariate measures related to the concept of synchronization (cp. Pikovsky et al., 2001) to EEG time series (e.g., Arnhold et al., 1999; Stam and van Dijk, 2002; Le Van Quyen et al., 2005; Ben-Jacob et al., 2007; Schevon et al., 2007; Stam et al., 2007; Ortega et al., 2008) in order to quantify interactions between two brain regions. Whereas these studies were predominantly focused on the analysis of epileptic activity, we investigated dependencies during different sleep stages. Using two analysis methods, we estimated strength and direction of interactions in EEG recordings from the following pairs of brain regions: RC–AH, RC–PH, PH–AH, NC–RC, NC–AH, NC–PH.

The directionality index (Rosenblum and Pikovsky, 2001) is a measure for directional influences based on phase
synchronization. In general, phase locking conditions can be defined for m:n phase relations (n; m ∈ N). Here, we restricted ourselves to the case of 1:1-synchronization; see Supplement A.1. The main idea of this approach is to model the phase dynamics of two systems using aligned Fourier series – for further remarks see Supplement A.2. The directionality index $d(1,2)$ was calculated via mutual influences which consist of derivatives of the modeled phase dynamics, and the index was normalized to the range between ±1. In the case of a positive directionality index, system 1 predominantly drives system 2, and in the case of negative values, system 2 drives system 1. If the directionality index approaches zero, coupling is bidirectional.

To extract phases from the EEG epochs, we used the Hilbert transform (Gabor, 1946; Panter, 1965), which approximates the phase from a broadband signal in a frequency adaptive manner – for additional (pre-) processing steps see Supplement A.4.

Since measures for directionality are generally restricted to weakly coupled systems, we discarded values of $d(1,2)$ corresponding to completely phase synchronized or uncoupled systems. In order to quantify the strength of coupling, we calculated the mean phase coherence (Mormann et al., 2000) (referred to as $R$) concomitant to the directionality index; see Supplement A.3. We used a double-sided threshold of $5\%$ as an exclusion criterion, i.e., directionality indices were included for $0.5 < R < 0.95$, which is a trade-off between a sufficient number of remaining epochs and the reliability of the directional estimate of a single epoch (Mardia, 1972; Smirnov et al., 2007; Osterhage et al., 2008). For all patients, 5367 (5004) epochs remained for estimating $d(1,2)$ and R after artefact inspection; the number of resulting directionality indices (after removing $d(1,2)$ values due to R-thresholds) averaged across all pairs of brain regions is denoted in brackets. Of these, 1404 (1281) epochs corresponded to waking state, 486 (444) to sleep stage 1, 1496 (1433) to sleep stage 2, 1380 (1321) to SWS, and 601 (525) to REM sleep.

### 2.3 Statistical analysis

The directionality index values were Fisher-$z$ transformed before further statistical analysis. For each patient, for each pair of brain regions, and for each sleep stage, $d(1,2)$ values were averaged across all epochs resulting in $d(1,2)$). We conducted a two-way analysis of variance (ANOVA) of $d(1,2)$ values with STAGE (awake, stage 1, stage 2, SWS, REM) and PAIR (RC–AH, RC–PH, PH–AH, NC–RC, NC–AH, NC–PH) as repeated measures. Further, we applied one-way ANOVAs for each pair with STAGE as repeated measures. P-values were Greenhouse-Geisser corrected (Greenhouse and Geisser, 1959) for inhomogeneities of covariance if necessary. The average over $d(1,2)$ across all patients for each pair of brain regions and each sleep stage is denoted as directional relationship $D(1,2)$.

### 3. Results

#### 3.1 Qualitative findings

Before investigating the influence of different vigilance states on the directional interactions between brain regions, we inspected a possible relationship between strength and direction of interaction. We could not observe a clear-cut relationship neither for different vigilance states nor between different brain regions. The correlation coefficients (Spearman’s rank; overall correlation coefficient $r = −0.08$) typically ranged around zero.

An exemplary time course for the directionality index $d(1,2)$ with the associated sleep stages is depicted in Fig. 1. The data from this patient indicate that the coupling tends to a predominant influence of AH onto NC during waking state. This influence, however, decreases towards sleep, particularly during SWS.

The directional relationships $D(1,2)$ for all combinations of brain regions and for all sleep stages are shown in Fig. 2. The direction of coupling between medial temporal sites qualitatively seems to depend little on sleep or waking state as we observed no clear directional reversals. Furthermore, AH shows predominant driving onto PH and RC. With regard to the coupling between NC and MTL, maximum directionality values suggest a driving of the NC by MTL structures during waking state. Towards deeper sleep stages, values of $D(1,2)$ indicate an increasing influence of NC onto MTL regions.
Fig. 1 – Top: Temporal evolution of directionality index $d^{(1,2)}$ with smoothed curve (adjacent averaging with 15 points) between AH and NC for one patient. Negative values of $d^{(1,2)}$ indicate predominant driving of AH onto NC, whereas values around zero indicate symmetric coupling. The corresponding sleep or waking states are depicted below.

Fig. 2 – Directional relationships between pairs of brain regions (denoted above) for different sleep and waking states. The error bars are standard errors of the means (s.e.m.). Positives values of $D^{(1,2)}$ indicate predominant influence of the first structure onto the second.
3.2. Changes of coupling direction

To investigate group effects of changes of coupling direction in dependency of waking or sleep states, we applied the statistical analyses described in the Methods section. A two-way ANOVA with STAGE and PAIR as repeated measures revealed a STAGE × PAIR interaction (p < .05; F_{20,180} = 3.234; ε = .152), but no significant main effects. This finding indicates a dependency of directional coupling on STAGE which differs for the different pairs of brain regions.

In a next step, one-way ANOVAs for the different pairs of brain regions with STAGE as repeated measures were computed, and revealed significant or near significant effects only for pairs of regions that included NC; see Table 2. Further, investigating the directional changes underlying these effects, we observed that the changes were significantly different between waking state and sleep, as well as between stage 1 and REM sleep (paired t-tests; p < .05); see Table 3. In all cases, we observed a decrease of driving of NC by MTL or even a predominantly driving influence from NC onto these regions during sleep as compared to wakefulness, as well as during REM sleep compared to stage 1. Taken together, our results suggest an increasing influence of NC onto MTL towards sleep.

3.3. Asymmetry of driver-responder relationships

Finally, we investigated the asymmetry of driver-responder relationships for all conditions (STAGE and PAIR; t-tests against zero; threshold of p < .05) and observed that only AH shows significant asymmetric driving; see Fig. 3. For at least one (awake or sleep) state, this brain region influences PH, RC, and NC above pure symmetric coupling. The other couplings may be considered bidirectional or not significantly asymmetric, but not as absent, due to corresponding coupling strengths: Values of R averaged across all epochs ranged between .26 and .67 for all patients, vigilance states, and pairs of brain regions. These values are significantly different from uniformly distributed phase values (p < .05; Rayleigh test of uniformity; Mardia, 1972).

4. Discussion

Our results indicate that directional dependencies between hippocampus and neocortex should be regarded as more complex than pure unidirectional influences inverting between waking and sleep states. In contrast, we observed that couplings were predominantly bidirectional. Particularly, the neocortex seems to exert an increasing influence onto structures of the medial temporal lobe towards deeper sleep stages. Nevertheless, we observed no significant asymmetric driving of MTL structures by the neocortex. Still, these findings differ from the predictions of the standard consolidation model. They are rather reminiscent of an alternative scenario (Tononi et al., 2006) as well as of several recent studies (Sirotta et al., 2003; Isomura et al., 2006; Mölle et al., 2006) with regard to the directionality changes. We have to emphasize that in the present exploratory study no controlled learning tasks were administered to the patients before sleep onset. Ideally, a follow-up investigation should directly relate directional coupling to memory performance as assessed by a task comprising pre-sleep encoding and post-sleep retrieval. Consolidation processes have been linked to the occurrence of slow oscillations (<1 Hz) during sleep (Born et al., 2006), which predominantly arise from prefrontal cortex (Massimini et al., 2004). Moreover, higher-order areas within association neocortex are supposed to be involved in consolidation processes (e.g., Born et al., 2006). Thus, besides a larger sample size, coverage of prefrontal, as well as association neocortex would be desirable for future studies.

The observed directional changes may be interpreted following the proposal of co-activation of neocortex and hippocampus during SWS (Ji and Wilson, 2007; Mehta, 2007), complex than pure unidirectional influences inverting between waking and sleep states. In contrast, we observed that couplings were predominantly bidirectional. Particularly, the neocortex seems to exert an increasing influence onto structures of the medial temporal lobe towards deeper sleep stages. Nevertheless, we observed no significant asymmetric driving of MTL structures by the neocortex. Still, these findings differ from the predictions of the standard consolidation model. They are rather reminiscent of an alternative scenario (Tononi et al., 2006) as well as of several recent studies (Sirotta et al., 2003; Isomura et al., 2006; Mölle et al., 2006) with regard to the directionality changes. We have to emphasize that in the present exploratory study no controlled learning tasks were administered to the patients before sleep onset. Ideally, a follow-up investigation should directly relate directional coupling to memory performance as assessed by a task comprising pre-sleep encoding and post-sleep retrieval. Consolidation processes have been linked to the occurrence of slow oscillations (<1 Hz) during sleep (Born et al., 2006), which predominantly arise from prefrontal cortex (Massimini et al., 2004). Moreover, higher-order areas within association neocortex are supposed to be involved in consolidation processes (e.g., Born et al., 2006). Thus, besides a larger sample size, coverage of prefrontal, as well as association neocortex would be desirable for future studies.

The observed directional changes may be interpreted following the proposal of co-activation of neocortex and hippocampus during SWS (Ji and Wilson, 2007; Mehta, 2007),
suggestions a coordinated replay in both structures, which could be an important feature of memory consolidation. According to this model, newly learned memory traces are integrated into neocortical regions during co-activation, while erasing recently learned information in the hippocampus at the same time (Mehta, 2007). The mechanism of coordinated replay fits well within the synaptic homeostasis hypothesis (Tononi and Cirelli, 2006). This hypothesis suggests that one important function of sleep is to downscale the synaptic strength to a baseline level in order to release synaptic capacities for the encoding of new experiences.

Additionally, we observed significant asymmetric dependencies, which reflect a predominant influence of the anterior hippocampal region onto the others. However, this asymmetry of coupling does not indicate pure unidirectional driving of the close-by and remote brain regions, because the largest coupling values are only in the order of \( \sim 2 \) (with \( \pm 1 \) representing pure unidirectional coupling). Nevertheless, one may assume that a rhythm generating structure in or nearby the anterior hippocampus influences other regions, which is supported by the theory of theta oscillation pacemakers in the CA1 or CA3 region (Buzsáki, 2002).

In general, we cannot exclude that our results may be biased by epileptiform activity or by antiepileptic medication of the patients. Because there are no intracranial recordings in healthy subjects, it is in principle impossible to investigate, whether EEG data obtained in epilepsy patients are comparable to those in healthy subjects. However, it has been shown for simple cognitive experiments (auditory and visual oddball paradigms) that event-related potentials from the non-focal side are qualitatively similar to intracranial recordings in healthy monkeys (Paller et al., 1992). There are hints for alterations of sleep-related processes due to epilepsy, e.g., the coupling between sleep ripple activity and slow oscillations seems to be affected by medial temporal pathology (Clemens et al., 2007). Moreover, alterations of the sleep structure due to epilepsy have been observed: Typically, there are more arousals due to seizures and the duration of REM periods is reduced in epilepsy patients (Bazil, 2000; Rocamora et al., 2008). However, there are rather no qualitative changes of sleep EEG due to epilepsy in the absence of seizures. In our study, we only investigated EEG recordings of nights without clinical seizures. EEG epochs were carefully inspected for epileptiform activity – even epochs containing a single epileptic spike were discarded leading to exclusion of 66.4% of epochs from further analysis. Furthermore, only electrode contacts, which were located at the non-affected side of patients suffering from clear-cut unilateral focal epilepsy, were included into analysis. The effect of antiepileptic medication on sleep depends on the chosen drug. For instance, several antiepileptic drugs seem to improve the sleep structure by reducing sleep latency and the number of awakenings (Rocamora et al., 2008). As patients in our study received highly variable medication depending on clinical circumstances (see Table 1), we can at least exclude that our effects depend on any specific single drug.

The directionality approach used in this study is well suited for analyzing oscillatory data, but it has some limitations. Analyses investigating frequency-specific dependencies and, also, \( n,m \)-relations between different frequency bands, would be desirable. However, phase-based directionality methods are still at an early stage of development. Whereas previous studies mainly focused on amplitude (cross-correlation), this approach only includes phase aspects. Information theoretical approaches, which are related to the concept of Granger causality, estimate directional dependencies including both amplitude and phase values (e.g., Babiloni et al., 2007; Brazdil et al., 2007; Supp et al., 2007; Staniek and Lehnertz, 2008). It remains an open question, which measures are best-suited to capture the neuronal mechanisms underlying functional interactions between brain areas.

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**Appendix**

**A. Supplement**

**A.1. Phase locking condition**

In the following, the extracted phase time series of two systems (1,2) are denoted as \( \phi_{1,2}(t) \), with \( t_j = n_t \Delta t \), the sampling interval \( \Delta t \), and the number of data points \( N \). If the systems are synchronized, the phase locking condition

\[
| n \phi_{1,2}(t_j) - m \phi_{1,2}(t_j) | \leq \text{const} \quad m,n \in \mathbb{N} \tag{1}
\]

has to be fulfilled. In this study, we restrict ourselves to the most intuitive case \( m = n = 1 \).

**A.2. Directionality index**

The increments of phase values of two signals can be calculated via \( \Delta_{1,2} = \phi_{1,2}(t_{j+1}) - \phi_{1,2}(t_j) \) with the phase time series \( \phi_{1,2}(t) \) and time increment \( \tau \). According to previous findings (Rosenblum et al., 2002), the time delay was chosen depending on the mean periods \( T_{1,2} \) of the oscillators as \( \tau = \frac{1}{\min (T_1, T_2)} \). In order to approximate the deterministic parts of the phase dynamics, we used finite Fourier series:

\[
F_{1,2}(\phi_{1,2}(t_j), \phi_{1,2}(t_j)) = \sum_{k,l} A_{1,2}^{l,m} \exp (ik\phi_{1,2}(t_j) + il\phi_{1,2}(t_j)). \tag{2}
\]

We followed Rosenblum and Pikovsky (2001) and included all Fourier terms, which fulfill a combination of summation indices: \( |k| \leq 3 \) for \( |l| = 0 \), \( |l| \leq 3 \) for \( |k| = 0 \), and \( k = l = 1 \).

The coefficients \( A_{1,2}^{l,m} \) were obtained by fitting \( F_{1,2} \) to the phase increments in the least squares sense. Therewith, the influence of one system onto the other can be defined as

\[
c_1^{1,2} = \int_0^{2\pi} \int_0^{2\pi} \frac{\partial F_{1,2}^{l,m}}{\partial \phi_{1,2}} \frac{\partial F_{1,2}^{l,m}}{\partial \phi_{1,2}} \, d\phi_1 \, d\phi_2, \tag{3}
\]

which can be solved analytically (Smirnov and Bezruchko, 2003) yielding

\[
c_1^{1,2} = 2\pi^2 \sum_{k,l} P(A_{1,2}^{l,m})^2. \tag{4}
\]
With these coefficients, the directional relationship between the two systems can be normalized to the interval [−1, 1]:

\[
d^{(1,2)} = \frac{C_2 - C_1}{C_1 + C_2}
\]

(5)

For \(d^{(1,2)} \rightarrow 1\), system 1 predominantly drives system 2, and in the case of negative values, system 2 drives system 1. If the directionality index approaches zero, coupling is bidirectional.

### A.3. Mean phase coherence

The mean phase coherence \(R\) (Mormann et al., 2000) estimates the coupling strength between two oscillatory systems (1,2) by quantifying the correlation between two phase time series \(\phi_1(t), \phi_2(t)\). The measure is defined as

\[
R = \frac{1}{N} \sum \exp \left( i(\phi_1(t) - \phi_2(t)) \right)
\]

(6)

and is confined to the interval [0, 1]. \(R\) approaches zero, if the systems are uncoupled. For fully synchronized systems, \(R \rightarrow 1\).

### A.4. Phase estimation

For each window, the data were demeaned, i.e., the Fourier coefficient with frequency \(\omega = 0\) was set to zero. Additionally, to avoid edge effects, we added a Hanning window (cosine half-wave) to 1/8 (2.5 sec) of the beginning and the end of each window, and after applying the Hilbert transform, 1/8 of the calculated phase values were discarded on each side of every window.

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