User Research
Sleep and functional imaging
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Abstract
To study the brain’s activity during sleep, functional MRI in combination with simultaneous polysomnographic recordings has been used in the last decade as a combination of methods with high spatial and temporal resolution. Recent data analysis tools have opened a window to determine cerebral functional networking in task-free fMRI data, ideally suited for an application in sleeping subjects. Data are discussed which are derived from complementary analyses. We describe how reorganization of functional cerebral communication may further our understanding of phenomena like fading of consciousness during sleep, and how information reprocessing during sleep may be linked to global flow of information in light sleep and more local reprocessing in deep sleep.

Introduction
For studying human sleep, polysomnographic recordings which include electroencephalography (EEG), electromyography (EMG) and electrooculography (EOG), are a prerequisite to objectify onset of sleep as well as different sleep stages. In 1968, Rechtschaffen & Kales (Rechtschaffen and Kales, 1968) published their guidelines how to identify different sleep stages, namely wake-sleep transition (sleep stage 1), light sleep (stage 2), stages 3 and 4 (slow-wave sleep (SWS)) and REM sleep. More recently, these criteria were revised by the American Academy of Sleep Medicine. In the EEG, non-REM sleep is characterized by occurrence of typical graphoelements like sleep spindles and K-complexes (their first occurrence defining sleep stage 2), and increasingly pronounced slow frequency fluctuations (0.5-2 Hz) in deep SWS. Further, rapid eye movement (REM) sleep represents an elusive sleep stage which shares some similarities with wakefulness, e.g. increased high-frequency EEG contributions as compared with non-REM sleep, but shows rapid eye movements and a strongly reduced muscle tone in the EMG reflecting the REM sleep specific blockade of skeletal muscle activity.

Neuroimaging research in sleep has long been the domain of radiotracer methods like positron emission tomography (PET) due to the easy combination with polysomnography. However, PET has some drawbacks: It is a costly technique with rather low temporal and spatial resolution, and exposure to ionizing radiation which limits serial measurements. In contrast, functional magnetic resonance imaging (fMRI), commonly available on most clinical MR systems, allows for repeated acquisition over several hours without any potential harm for volunteers. Furthermore, higher spatial and temporal resolution allows for a more detailed analysis of cerebral activity, up to the point of fast paced EEG events occurring during sleep, e.g. spindle activity, K-complexes or rapid eye movements.

Combining fMRI with polysomnography has been challenging for a long time. Most of all, the noisy experimental condition during sampling of MR images creates an environment which is not sleep inducing. Furthermore, as compared with other simultaneous fMRI/EEG studies, the subject’s comfort needs to be particularly considered, as the volunteer has to remain motionless in a supine position during an extended period of time, which may lead to irritation and pain mostly at occipital electrode positions. Furthermore, it is not possible to remount electrodes during the experiment without repositioning the subject – which certainly would lead to an awakening. Therefore, long term stability of the electrode montage and stability of electrode impedances has to be guaranteed.

At the Max Planck Institute of Psychiatry, we have performed our first combined EEG/fMRI experiments in sleeping subjects in the year 2000. At that time, commercially available MR-compatible EEG systems had just been introduced. Our first system was built by the company Schwarzer, and used individual electrode cables (32 channels) usable for EEG channels, EOG, EMG and ECG for polysomnography. Also, MR artefact correction was in its infancy these days. With the Schwarzer system, offline correction for gradient-artefacts was based on notch-filtering of the affected frequency bands. Because of the limited capacity of this type of correction algorithm as well as in an attempt to reproduce PET results in sleeping subjects, that were acquired in a silent environment, we and others initially focussed on ‘silent’ fMRI protocols. While Lövblad et al. used a BURST sequence (Lovblad et al., 1999) with only very few gradient switching points, we focussed our efforts on a silent gradient echo sequence with softly changing gradient flanks (Czisch et al., 2002). Beside the reduced sound pressure (in fact, the noise of the magnet’s Helium-pump was louder than the noise by fMRI data acquisition), we took benefit of the Schwarzer system, offline correction for gradient-artefacts was based on notch-filtering of the affected frequency bands. With the Schwarzer system, offline correction for gradient-artefacts was based on notch-filtering of the affected frequency bands. Because of the limited capacity of this type of correction algorithm as well as in an attempt to reproduce PET results in sleeping subjects, that were acquired in a silent environment, we and others initially focussed on ‘silent’ fMRI protocols. 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preferen of EPI acquisition. If one is willing to accept high drop-out rates due to subjects unable to fall asleep, this method is superior in terms of spatial and temporal resolution of the MR data acquired. Today, all research sites studying sleep under fMRI conditions are focussing on this method. Here, gradient induced artefacts are much more severe than in silent methods. In 2005, we changed our EEG hardware to an MR compatible 32-channel system by Brain Products, with customized EEG caps modified for polysomnographic recordings (EasyCap, 25 EEG, 3 EMG, 3 EOG, 1 ECG channels). Achievements in Brain Product’s EEG hardware by introduction of MR scanner synchronized data collection along with higher rate of data sampling (5 kHz), improved amplifier characteristics and sophisticated artefact correction algorithms implemented in the BrainVision analyzer software nowadays allow for collection of high-quality polysomnographic recordings during fMRI. Online artefact correction using BrainVision RecView further allows for identification of specific sleep stages during the experiment, which is especially important when REM sleep is targeted: Some MR scanners are only capable to store a limited amount of image data per run. Actual data collection therefore needs to be restricted to the sleep stage of interest, but a constant scanner noise background is necessary from the beginning to allow the subject to habituate, to fall asleep and to pass through non-REM sleep stages before REM sleep occurs. This can be achieved by running the MR system in a ‘dummy’ mode without actual data sampling, but with all other features like scanner noise and vibrations preserved, unfortunately also including gradient induced EEG artefacts.

Using combined EEG/fMRI experiments, our lab and colleagues from few other neuroimaging sites worldwide have successfully investigated the functional correlates of EEG features like sleep spindles (Schabus et al., 2007), K-complexes (Czisch et al., 2009) and slow waves (Mascetti et al., 2011) in non-REM sleep, as well as of rapid eye movements (Wehrle et al., 2005) in REM sleep.

In the following, we will describe recent advances in fMRI data analysis which aim at identifying sleep stage specific alterations in interregional cerebral communication, termed functional connectivity analysis (for a detailed description please refer to the original publications). This approach exploits temporally correlated signal fluctuations in fMRI time courses of distinct functionally connected brain regions, that are referred to as ‘resting state networks’ (RSNs) due to their spontaneous presence in the absence of an external task during data acquisition. During wakefulness, several such networks have been described in depth, including primary sensory networks, networks sustaining higher cognitive functions, and the so called “default mode network” (DMN). The DMN was first identified in a meta-analysis of PET studies, showing the remarkable feature of being more active when a subject is not engaged in task performance. In fMRI, the DMN can evenly be observed as task-negative network in experiments when a subject is not engaged in task performance. In fMRI, the DMN shows increased task-related activity. Therefore, the DMN has been attributed to support internal awareness, while its counterpart, the anti-correlated network (ACN), sustains external awareness. In human sleep, both internal and external awareness are obviously reduced. We therefore hypothesized that functional network alterations should be detectable in sleep fMRI studies. The DMN and ACN can also be detected under resting state conditions, without any task, based on independent component analysis (ICA) or on cross-correlation analysis of all brain voxels with the time course of a given seed region.

Using a population of 25 young healthy volunteers, we acquired 30 minutes of whole brain fMRI data at 1.5 Tesla while the subjects were falling asleep in the MR scanner. If the subjects did not fall asleep or did not reach all sleep stages (stage 1 and 2, and SWS) the experiment was repeated. From these data, 5 minute epochs were extracted which had to represent a single sleep stage for more than 85% of the time. All subjects succeeded in falling asleep, and a total of 40 fMRI runs were performed. From this data pool, 93 epochs of a single sleep stage were extracted. 27 epochs during wakefulness, 24 during sleep stage 1, 24 during stage 2 and 18 in SWS were forwarded for final analysis. After appropriate preprocessing, data were subjected to ICA which resulted in 20 components for wakefulness, 18 in stage 1, 18 in stage 2 and 15 in SWS. 10 components for each stage were selected according to a previously published procedure. The present work focuses on data analysis limited to components with significant correlations between anterior and posterior nodes of the DMN as well as of the ACN during NREM sleep (Fig. 1).

Figure 1: Default-mode network (DMN) connectivity (hot colors) with anti-correlated regions (cool colors) throughout wakefulness (A), light sleep stage 1 (B), sleep stage 2 (C), and slow-wave sleep (D), with t-statistics (E) and temporal dynamics (F). Light sleep was characterized by a reduced contribution of the para-hippocampal gyrus (PHG) to the DMN and with reduced anti-correlations, although DMN connectivity was generally maintained. Slow-wave sleep (SWS) was marked by a breakdown in correlations between anterior and posterior nodes of the DMN, and further tests revealed that these between-stage differences were significant at pcorr < 0.05. PCC/RspC: posterior cingulate cortex/retrosplenial cortex; mPFC: medial prefrontal cortex; IPL: inferior parietal lobule; DAS: dorsal attention system; ITG: inferior temporal gyrus; PHG: (para-)hippocampal gyrus; Th: Thalamus; sgACC: subgenual anterior cingulate gyrus; STG: superior temporal gyrus. From Sämann et al., 2011.
First, we found that as already as in sleep stage 1, the extended hippocampal formation (HF+) drops out of the network, which suggests a different role of (para-)hippocampal activity in wakefulness and during sleep. Second, the PCC/RspC node gradually lost its connectivity to the medial prefrontal cortex, and also shows reduced intra-node connectivity strength. Considering the role of this brain area in the network of consciousness, as e.g. shown by studies on loss of consciousness during anaesthesia, the disintegration of the PCC/RspC node may in part explain the loss of conscious awareness as experienced during sleep. Also the ACN shows increasingly reduced intra-network functional connectivity in the descent to sleep. Finally, the strict dichotomous nature of the DMN/ACN anticorrelation get lost, suggesting independent residual fluctuations of these two networks during sleep.

The finding of a dropout of hippocampal formation from the DMN raised the question of the fate of the hippocampal functional connectivity during sleep. To answer this question we performed a seed-based analysis on the same data pool using the hippocampus and its subregions as a ROI (Andrade et al., 2011). This revealed that HF+ functional connectivity which is bound to the DMN in wakefulness, disintegrates from this network in stage 1, and builds up different connectivities to neo-cortical regions, especially during sleep stage 2. Given the fact that HF-cortical interplay has been proposed to constitute a hallmark of sleep dependent memory consolidation during deep sleep, the increased connectivity during light sleep stage 2 was surprising. The most wide-spread connectivity pattern was observed for the subiculum subregion of the HF+ formation, that represents the output region of the hippocampus, with strong connectivity to frontal, temporal and occipital areas. We further detailed the influence of cortical sleep spindles on HF+ functional connectivity. Fast (13-15Hz) as well as slow (11-13Hz) spindles were detected in the EEG recordings during sleep stage 2, and fMRI correlations of fast spindles were as reported (Schabus et al., 2007). The appearance of fast spindles, however, was not directly coupled to increased hippocampal BOLD signal increase, which was unexpected given preclinical observations of synchronized cortical spindle and hippocampal ripple activity. Remarkably, it was functional connectivity between the HF+ and neocortical areas that increased during the appearance of fast spindles, as pinned down by a whole brain interaction analysis of fast spindles and HF+ BOLD signal fluctuations. This finding may provide a functional correlate of such synchronized hippocampal ripple and cortical spindle activity (Fig. 2).

**Functional connectivity and network analyses**

A new analysis method, referred to as graph theoretical analysis, has recently been applied to sleep data in our lab (Spoormaker et al., 2010). In this analysis, the brain is parcelled into numerous regions of interest using standard digital atlas systems. We used the AAL atlas, which provided us with 90 cortical and subcortical ROIs. Preprocessing of the above mentioned data pool included wavelet filtering to restrict the temporal fluctuations of the regional BOLD time series to the frequency band below 0.1Hz. Cross-correlation matrices were then calculated and correlation coefficients thresholded to derive a binary connection matrix. It became evident that compared with wakefulness, sleep stage 1 is characterized by a loss of thalamo-cortical connectivity, but at the same time showed increased cortico-cortical coupling (Fig. 3).
Stage 2 was comparable to stage 1, but SWS was characterized by a breakdown of long-range functional connectivity and the emergence of more locally connected clusters. Graph theoretical analyses generally allows for condensing the complex network topology to a few characteristic parameters, e.g. path length and local clustering coefficients. An optimal neural network adopts a ‘small world’ topology, with high local clustering but also presence of so called hubs, i.e. few relay stations which allow for rapid transfer of information throughout the whole network. This small-worldness was generally preserved throughout all NREM sleep stages, but local clustering increased and path length decreased in SWS, signifying a network with only limited capacity to integrate information on a global level, while still allowing for information processing in local cerebral subregions.

Conclusions

Modern approaches to fMRI analysis may complement each other as each one of them reveals specific aspects of cerebral network organization. Our data disclose a functional reorganization of cerebral network activity during human NREM sleep. The disintegration of the DMN already during wake-sleep transition (which can similarly also be noted under increased sleep pressure such as after partial sleep deprivation (Sämann et al., 2010)) informs researchers to consider vigilance fluctuations during resting state fMRI experiments, and ideally to monitor vigilance using EEG. Due to the simplicity of the experimental setup in such experiments, resting state networks, especially the DMN, have been studied in numerous psychiatric and neurological patient populations. It is thus important for clinical studies to consider the potentially strong confounding effects of vigilance fluctuations that may be different between populations, e.g. due to comorbid sleeping disorder, and which may lay the ground for wrong conclusions on disease states. Our data on altered functional hippocampal connectivity pattern especially during sleep stage 2, along with the findings of increased local clustering in slow-wave sleep, may indicate sleep stage specific contributions to memory consolidation processes, with increased capacity of information transfer between brain regions in stage 2, and optimal local reprocessing of such information during SWS. Regarding loss of conscious awareness as experienced in sleep, our data suggest several mechanisms: Breakdown of thalamo-cortical functional connectivity in the wake-sleep transition likely reducing external stimulus transmission, as well as disintegration of long-range functional connectivity resulting in loss of capacity for global information integration. Along with general decoupling of the thalamus from the cortex, altered connectivity within the prefrontal/retrosplenial cortex, and of this area with anterior DMN nodes, presumably represent functional correlates of loosing consciousness in the decent to sleep, a view largely supported by lesion studies and studies on anaesthesia that have identified this connectivity hub as a key regulatory site of wakeful consciousness. From a clinical perceptive, combined fMRI/EEG studies may thus help to elucidate similarities and differences between loss of consciousness under sedation and during natural sleep.

References


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