Development of the Brain’s Default Mode Network from Wakefulness to Slow Wave Sleep

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Falling asleep is paralleled by a loss of conscious awareness and reduced capacity to process external stimuli. Little is known on sleep-associated changes of spontaneously synchronized anatomical networks as detected by resting-state functional magnetic resonance imaging (rs-fMRI). We employed functional connectivity analysis of rs-fMRI series obtained from 25 healthy participants, covering all non-rapid eye movement (NREM) sleep stages. We focused on the default mode network (DMN) and its anticorrelated network (ACN) that are involved in internal and external awareness during wakefulness. Using independent component analysis, cross-correlation analysis (CCA), and intraindividual dynamic network tracking, we found significant changes in DMN/ACN integrity throughout the NREM sleep. With increasing sleep depth, contributions of the posterior cingulate cortex (PCC)/retrosplenial cortex (RspC), parahippocampal gyrus, and medial prefrontal cortex to the DMN decreased. CCA revealed a breakdown of cortico-cortical functional connectivity, particularly between the posterior and anterior midline node of the DMN and the DMN and the ACN. Dynamic tracking of the DMN from wakefulness into slow wave sleep in a single subject added insights into intraindividual network fluctuations. Results resonate with a role of the PCC/RspC for the regulation of consciousness. We further submit that preserved corticocortical synchronization could represent a prerequisite for maintaining internal and external awareness.

Keywords: consciousness, DMN, fMRI, functional connectivity, sleep

Introduction

Among the spontaneously synchronized neuronal networks detectable by resting-state functional magnetic resonance imaging (rs-fMRI), the default mode network (DMN) has been characterized in most depth (Raichle et al. 2001; Fox et al. 2005; Buckner et al. 2008). It spans the bilateral posterior cingulate cortex (PCC) and retrosplenial cortex (RspC), inferior parietal lobule (IPL), medial prefrontal cortex (mPFC), parts of the hippocampal formation (Raichle and Snyder 2007; Buckner et al. 2008), and the temporal lobe. The latter 2 regions have not been consistently been reported, suggesting a loose integration in the DMN. Synchronous fluctuations below 0.1 Hz with blood oxygen level-dependent (BOLD) amplitudes in the magnitude of task-induced activations (1–3%) are further characteristics of the DMN (Damoiseaux et al. 2006).

The DMN has first been observed as a task-negative network, showing increased metabolic demand during the “baseline” condition and has therefore been hypothesized to reflect intrinsic default brain processes (Shulman et al. 1997; Raichle et al. 2001). Furthermore, the DMN is preferentially “active” when individuals are not focused on the external environment but engaged in intrinsic activity such as autobiographical memory and future envisioning (Gusnard et al. 2001; Buckner et al. 2008). It is thought by some to consist of subsystems involved in memory collection and building of associations, flexible use of this information to construct mental simulations, and integration of these processes (Buckner et al. 2008). It is further intrinsically coupled to an anticorrelated network (ACN) (Fox et al. 2005; Fransson 2005, 2006) that typically comprises regions activated during goal-directed task performance, including the intraparietal sulcus, frontal eye fields, middle temporal area, the supplementary motor area, and insula (Cabeza and Nyberg 2000; Fox et al. 2005). Subregions of this “task-positive” network overlap with the dorsal attention system (such as the intraparietal sulcus, frontal eye fields, and supplementary motor area) and with the ventral attention system (such as the insula) that both mediate different aspects of externally directed attention (Corbetta and Shulman 2002; Fox et al. 2006; Boly et al. 2008). It was further demonstrated that the anterior and posterior midline node of the DMN are linked to 2 separable ACNs (Uddin et al. 2008).

As the various cognitive states that activate the DMN mostly represent internally directed awareness processes up to the level of “self-awareness,” the DMN has been conceptualized as antagonistic in relation to the ACN that supports functions of external awareness (Boly et al. 2008). Against these dichotomic features of the DMN and ACN that were deduced from data obtained during wakefulness, their interaction with sleep is of particular interest as sleep is characterized both by reduced capacity to process external information and by reduced self-awareness (Hobson and Pace-Schott 2002). Previous imaging studies of spontaneous neural activity during sleep mostly have contrasted signal averages of different sleep stages: With slowing of the electroencephalography (EEG) during the process of falling asleep (Steriade et al. 1993), metabolic activity decreases in various brain regions, most pronounced in frontal areas (Braun et al. 1997; Maquet 2000; Dang-Vu et al. 2005; Kaufmann et al. 2006). Further, BOLD responses to external stimuli during sleep are categorically different from those during wakefulness (Born et al. 2002; Czisch et al. 2002, 2004; Tanaka et al. 2003). Evidence for altered interaction between different brain regions in sleep also comes from high-density EEG studies where transcranial magnetic stimulation has been applied to investigate the propagation of focal cortical activity. While during wakefulness activity rapidly spreads over the cortex, such propagation was either absent in (non-rapid) eye movement (NREM) sleep or showed a more stereotypical global wave behavior (Massimini et al. 2005). Such reduced
corticocortical interaction is well compatible with the reduced integration of sensory information observed during sleep. Results were also interpreted as a breakdown of the thalamocortical system into unrelated modules, eventually causing the fading of consciousness during sleep (Tononi and Massimini 2008).

So far, few fMRI/EEG studies have analyzed spontaneous network organization during sleep: during light sleep, the DMN—as approximated by PCC seed correlation—was still detectable, as were spontaneous BOLD fluctuations of the visual cortex (Horovitz et al. 2008). Recent data reported by Larson-Prior et al. (2009) based on seed analysis showed preserved functional connectivity in 5 resting-state networks that included the DMN during sleep stage 2. Both studies highlighted the general persistence of DMN into sleep; however, sample sizes might have been too small to detect significant differential effects. Recently, Horovitz et al. (2009) reported reduced functional connectivity between nodes of the DMN in deep NREM sleep compared with wakefulness.

Similar to sleep, anesthetia also leads to reduced consciousness. During light sedation with midazolam, the DMN architecture as a whole was maintained; yet, contributions of the PCC to the DMN were decreased (Greicius et al. 2008). Even in anesthetized monkeys, the DMN can be observed as a functional unit (Vincent et al. 2007) that argues against the DMN being specific to humans or its persistence being a sufficient condition for consciousness. Structural integrity of the DMN across species and vigilance states may in part be based on direct white matter tract connections between its cortical nodes (Skudlarski et al. 2008; van den Heuvel et al. 2008; Greicius et al. 2009). In deeper anesthesia, reduced metabolic activity of the thalamus and the PCC is a common finding (for review, see Alkire and Miller (2005), and reduced connectivity of the PCC to the PFC has been reported from vegetative state patients (Laureys et al. 1999, 2004). Several clinical observations including lesion studies have further substantiated that the PCC/RspC and adjacent precuneal cortex play a key role in the neural network of consciousness (Vogt and Laureys 2005).

Against this background, we performed an fMRI/EEG study to investigate the DMN and its ACN during physiological vigilance changes. Statistical inference was based on complementary methods of functional connectivity analyses (independent component analysis [ICA] (Kiviniemi et al. 2003; Beckmann and Smith 2004) and CCA) in data obtained from 25 healthy volunteers during wakefulness, light NREM, and deep NREM sleep including slow wave sleep (SWS). We tested the following hypotheses. 1) The DMN as one functionally coherent network can be detected throughout all NREM sleep stages. 2) Connectivity among DMN nodes and between the DMN and ACN should decrease in parallel with deepening of sleep. Particularly, reduced contribution of the posterior node and disconnection of the mPFC are expected signifying reduced spontaneous corticocortical communication with fading consciousness. 3) Given that the parahippocampal gyrus was found less strongly connected to the posterior DMN node in previous studies, we hypothesized these nodes to be least robust toward vigilance changes. 4) Last, to dismiss the possibility that DMN changes are an artifact of our group stratification and reflect general between-subject rather than sleep stage dependent differences, we employed both an ICA-based method (recursive ICA) and a seed-based method (recursive seed correlation) to continuously track the DMN throughout a 26.7 min recording of an individual that passed through all vigilance stages in one session.

Methods

Subjects

The study protocol was approved by the local ethical committee, and participants gave their written informed consent prior to inclusion. Subjects underwent a general medical and structured psychiatric interview, clinical MRI, and EEG to exclude neuropsychiatric condition. Combined fMRI/polysomnography was obtained from 25 healthy adults (13 men, mean [standard deviation] age 24.7 [3.2] years and 12 women, 24.8 [2.5] years). Subjects were instructed to follow a regular sleep-wake schedule with bedtimes between 11 PM and 8 AM during the week prior to the experiment, documented by sleep diaries. They were asked to get up about 3 h earlier on the experimental day to increase the probability of falling asleep in the MRI scanner. Actigraphy during the night and day before the experiment was employed to control subjects’ keeping to the sleep restriction. Experiments started around 9 PM; after EEG montage and positioning in the MRI scanner, subjects were informed that no further active participation was necessary the following 2–3 h and that they could fall asleep. We did not mention that actually runs of 26.7 min were planned, as based on our experience with fMRI studies during sleep a rather vague instruction is more useful to avoid any expectation pressure to fall asleep.

fMRI and EEG Acquisition

Simultaneous polysomnography comprised 19 EEG electrodes placed according to the international standard 10/20 system, electrocorticogram, submental electromyography, and an electrocardiogram (sampling rate 5 kHz, EasyCAP modified for sleep, VisionRecorder Version 1.03, BrainProducts3). For noise protection, subjects wore ear plugs and headphones. fMRI was carried out at 1.5-T (Signa LX) using an 8-channel head coil. One fMRI run consisted of 800 functional whole-brain images (echoplanar images (EPI), repetition time 2000 ms, echo time 40 ms, flip angle 90°, 64 x 64 matrix, in-plane resolution 3.4 x 3.4 mm², slices 25, slice thickness 3 mm, gap 1 mm, oriented along anterior commissure-posterior commissure) acquired over 26.7 min. This time restriction originated in an absolute scanner software limitation to continuous acquisition of 20,000 slices at maximum. If the subject was not able to fall asleep, only reached light NREM sleep stages or showed an early and rapid transition to S2 and SWS as decided based on the online EEG assessment during the first run, a further acquisition was appended to increase the probability of recording the missing sleep stages. To allow for decisions during the recording session, the sleep stage was determined online by the experimentators with real-time gradient EEG artifact correction performed using the Vision Recorder (Version 1.03.0003) and Vision RecView (Version 1.0) software (Brain Products). In total, 40 fMRI runs of each 26.7 min length were acquired from 25 subjects (Supplementary Table 1).

Polysomnography Artifact Correction, Sleep Stage Rating, and Definition of Sleep Epochs

Simultaneous sleep recordings were corrected for gradient-induced and cardiorespiratory artifacts using the VisionAnalyzer 1.05 (Brain Products) before spectral filtering (0.5–30 Hz). Sleep stage scoring of all 40 fMRI runs was performed following Rechtschaffen and Kales (1968) criteria in frames of 20 s. Last, review of the 40 hypnograms was performed, and 93 epochs of 5 contiguous min with one clearly prevailing (>95%) vigilance stage and without arousals were defined (Supplementary Table 1).

Preprocessing and ICA

Functional images were processed using SPM5 (http://www.fil.ion.ucl.ac.uk/spm, FSL3.2 (http://www.fmrib.ox.ac.uk), MIRcro (http://www.spf.sc.edu/comd/roden/micro.html), and in-house software. Image preprocessing comprised 8 steps: 1) slice-time difference correction and affine motion correction performed on 800 images of each fMRI run, 2) interpolation of resulting images to native space and resolution, 3) concatenation of each of the 93 sleep epochs...
(represented by each 150 images) into a 4D file. 4) brain extraction to remove nonbrain tissue, 5) high-pass filtering (wavelength cut-off 100 s), 6) spatial smoothing (Gaussian kernel, 6 x 6 x 6 mm$^3$), 7) voxelwise demeaning of the data, and 8) normalization of voxelwise variance. Preprocessing steps 4-8 are part of the FSL-based ICA pipeline implemented MELODIC tool (version 3.2). Last, preprocessed data was whitened and projected into a 25-dimensional subspace using probabilistic principal component analysis where the number of dimensions was defined a priori. The whitened observations were decomposed into a set of autoregressive coefficients and spatial maps by optimizing for non-Gaussian spatial source distributions using a fixed-point iteration technique (Hyvarinen 1999). Estimated component maps were divided by the standard deviation of the residual noise to gain Z maps (Beckmann and Smith 2004), with positive and negative Z values corresponding to positive and negative correlation with the time course of the component.

**Automated Identification of the DMN/ACN**

All 25 Z maps of each epoch were spatially normalized to atlas space using the distributed EPI template of SPM5 (Montreal Neurological Institute space; trilinear interpolation, 2 x 2 x 2 mm$^3$). To identify the DMN observer independently, we employed a 2-step procedure as previously used (Greicius et al. 2007): 1) components with frequencies >0.1 Hz constituting more than 50% of the total spectral power were excluded as DMN fluctuation frequencies typically peak in the 0.05–0.1 Hz range and 2) remaining candidates were compared against a binary DMN template mask (sum of unthresholded Z values within the template area minus sum of Z values outside the template area) (Supplementary Fig. 2B). The highest ranked component was accepted to most likely represent the DMN of the epoch; to avoid false rejections of potentially changed DMN, the 5 highest ranked components were immediately stored for further analyses. The ACN is intrinsically represented in the same maps as negative Z values.

**Changes of Local DMN Contribution per Individual Sleep Stage**

The highest ranking Z map of each epoch was forwarded to voxelwise estimation of sleep-stage-dependent changes of the Z-scores in atlas space using one-way analysis of variance (4-level factor sleep stage). Estimation was performed under consideration of violation of sphericity as implemented in SPM5 (Friston et al. 2005). Analyses were confined to gray matter as defined by a lenient mask in atlas space. First, we implemented in SPM5 (Friston et al. 2005). Analyses were confined to gray matter as defined by a lenient mask in atlas space. First, we

**Dynamic Tracking of DMN Development in a Single Subject**

To evaluate if sleep-related DMN changes defined from our group analyses can be continuously tracked at the individual level, we followed the evolution of the DMN over 26.7 min in the recordings of an individual that passed through all sleep stages. As for the group analysis, we employed both an ICA-based and a seed-based method to visualize the DMN evolution over sleep stages. 1) "Recursive ICA" (Supplementary Fig. 1B): for this, ICA was first cycled 650 times throughout the entire recording of the individual using a sliding window that covered 150 images (i.e., 5 min) with step one image; from each cycle, 5 candidate components possibly representing the DMN were extracted as described above. Second, based on this preliminary 650 x 5 array of DMN candidates, a continuous DMN stream was identified by automatically comparing the current DMN at time t to the 5 candidate components of the next cycle at time t + 2s. Similarity was quantified with the Pearson's correlation coefficient r between Z maps of time points t and t + 2s, as recently suggested (Wang and Peterson 2009). Outliers, the average of the newly selected component and the 4 previous cycles served as template for the next cycle. 2) "Recursive seed correlation" (Supplementary Fig. 1B) to ensure that the dynamic tracking results are not specific to the ICA method, we also employed seed correlation analysis to define the DMN. As seed area, we defined the posterior midline hub of the initial DMN of the individual thresholded at Z > 10 (106 voxels). In a sliding window technique over 650 cycles, the average time course of this seed area was extracted and regressed on the 150 images of the respective time window. Whole-brain T maps of the correlation with the seed area were estimated using simple regression analysis and stored for each cycle.

The resulting collections of each 650 images of either tracking method (Z maps and T maps, respectively) were processed further to animated graphics (Supplementary Methods).

**Results**

**Subjects Sleep-Wake Schedule, Actigraphy Readout, and Sleep Pattern during fMRI**

All subjects demonstrated their sleep diaries and confirmed compliance to the recommended sleep–wake schedule in the week before the experiment. Actigraphy confirmed subjects’
keeping to the sleep restriction. Sleep latency (time until first appearance of S2), only considering the first attempt of the subject to fall asleep, was 7.6 ± 4.9 min. Over all 40 MRI runs, the average time spent in W, S1, S2, and SWS were 6.8 ± 5.2, 5.9 ± 4.2, 10.0 ± 5.4, and 4.8 ± 5.8 min, respectively. Eventually, hypnogram review identified 93 epochs (27, 24, 24, and 18 epochs of W, S1, S2, and SWS of 15, 18, 10, and 11 subjects) of each 5 contiguous min with a single prevailing vigilance stage, that is, less than 14% of time spent in flanking sleep stages and without arousals. Assignment of all 5-min epochs to subjects and runs including a description of the prevalence of the different sleep stages per run is detailed in Supplementary Table 1. No occurrence of REM sleep was observed that is not unexpected as our data were obtained only from the initial part of the sleep cycle and as REM sleep is suppressed in the noisy MR environment (Czisch and Wehrle 2009).

**DMN/ACN Configuration during Wakefulness**

In the EEG validated awake state, the DMN spanned the midposterior node (PCC, RspC, precuneus), lateral parietal areas (mainly in the IPL), ventral and dorsal mPFC, smaller bilateral inferior (ITG) and superior temporal gyrus (STG), and temporoamial areas (Fig. 1A). The latter mainly contained the PHG including the entorhinal cortex and a small proportion of the hippocampus proper. In addition, the subgenual anterior cingulate cortex and an anterior thalamic area were included (Supplementary Table 2). Significantly anticorrelated signal was observed in the bilateral IPL (bordering the supramarginal gyrus and STG), in the bilateral insula (bordering the inferior frontal gyrus and STG), and in the left lateral PFC (Fig. 1A) (Supplementary Table 2). This network is compatible with reported brain areas anticorrelated to the DMN in the resting state (Fox et al. 2005; Fransson 2006; Uddin et al. 2008; Sämann et al. 2010).

**Sleep-Stage-Dependent Changes of the DMN (ICA-Based Results)**

Fluctuation frequencies were in the typical range below 0.1 Hz for all sleep stages with spectral power changes found in the 0.1-0.15 Hz range (P < 0.05/25 = 0.002); yet no differences found below 0.1 Hz (Fig. 1F). Total DMN strength as defined by the sum of the Z scores within the DMN template area was higher during wakefulness compared with all sleep stages (all P < 0.001) (Supplementary Fig. 2C). Sleep-stage–specific analysis of the DMN/ACN at the group level (Fig. 1B–D) and analysis of differences between sleep stages (Table 1, Fig. 2) demonstrated converging results. The midposterior DMN node showed a marked decrease to moderate, stable levels reached in S1 with lower levels reached in a more anterior PCC cluster compared with the PCC/RspC cluster. The mPFC showed a stepwise decrease (Figs 1A and 2A), stabilizing at low but still significant levels with dispersed subclusters in SWS (Fig. 1D). In contrast, the bilateral PHG showed a stepwise decrease to the level of no significant contribution in S2. Relative deep decrease between S1 and S2 was found for the bilateral DMN node located in the inferior temporal gyrus (Fig. 1) with only the right-hemispheric node reaching significance in the differential contrast (Fig. 2A). The bilateral STG showed transient contribution to the DMN strongest during S2, with the left STG also appearing in the differential contrast (Figs 1C and 2A). The contribution of the bilateral ITG decreased between S1 and S2 (Figs 1A, B and 2A) with the right ITG appearing in the differential contrast (Figs 1C and 2A). The ACN retreated stepwise between W and S2 (Fig. 1C). This effect translated to the W < sleep comparison (Fig. 2B), yet, not to the differential F contrast. No significant changes of the bilateral lateral parietal DMN areas were detected (Fig. 2B). Bilateral superior and transversal temporal gyrus areas not involved during wakefulness reached significance selectively in S2 (Fig. 1C).

**Sleep-Stage-Dependent Functional Connectivity Changes between DMN/ACN Nodes**

Analysis of coherence of temporal BOLD time series of all selected nodes corroborated that functional connectivity between DMN/ACN nodes undergoes systematic, vigilance dependent changes (Fig. 3). This effect was detectable in both hemispheres and most pronounced between the anterior and posterior nodes of the DMN (all Pcorr < 0.0009). Equally, connectivity between the PCC/RspC with the PHG and the IPLDMN differed between sleep stages (all Pcorr < 0.006 and Pcorr < 0.013, respectively). PCC/RspC-IPLDMN connectivity decreased in S1 and S2 and was restored to levels not significantly different from wakefulness in SWS in the respective post hoc test. Transhemispheric coherence of corresponding DMN nodes was stable with the exception of left and right PCC/RspC that showed a slight increase in connectivity (Pcorr = 0.027). Furthermore, anticorrelation of ACN nodes with respect to DMN nodes decreased in general. More specifically, the bilateral IPLACN demonstrated reduced anticorrelation with the anterior and posterior midline DMN nodes whereas the bilateral insula and left lateral PFC of the ACN was strongly decoupled from the mPFC but showed relatively preserved anticorrelations (ρ ~ 0.150) with the PCC/RspC. Connectivity among the ACN nodes was significantly altered in about half of the connections (Fig. 3B); yet, intra-ACN correlations remained positive except for the left lateral PFC node. Transhemispheric connectivity decreased for the IPLACN (Pcorr = 0.030) and was unchanged for the insula. Generally, changes were into the direction as suggested by ICA, for example, showing decreasing functional connectivity between anterior and posterior midline nodes of each hemisphere between W, S1, and S2 (see Supplementary Tables 3a–f for post hoc comparisons and color codes in Fig. 3B for direction of connectivity changes).

**Dynamic Tracking of DMN Development in a Single Subject**

Both the ICA-based and the seed-based dynamic tracking of the DMN revealed that key findings of the group analysis can be detected at the individual level (Supplementary Animated Graphics). 1) Throughout all vigilance stages, the DMN was represented among the resting state networks gained by ICA, and it was extractable using the seed based method. 2) PHG contributions retreated as early as in S1. 3) Positively correlated mPFC and frontopolar areas were more strongly represented during wakefulness but remained detectable throughout all sleep stages. 4) The PCC and inferior RspC of the midposterior DMN node decreased in strength, reaching negative Z values in S2 and SWS. 5) Lateral parietal areas were largely stable across all sleep stages in both subjects. 6) During S2, transient contributions of middle and superior temporal gyrus were observed. During the transition from wakefulness to S1,
recursive ICA further demonstrated that competing DMN candidate components increased their similarity, compared with the initial unique DMN (Fig. 4A). During S2 and SW, a partial recovery of this change was observed (Fig. 4B).

Discussion

We aimed at providing a systematic description of vigilance dependent changes of the DMN and its anticorrelated counterpart (ACN) that overlaps with parts of the dorsal and...
ventral attention network, employing parallel EEG/fMRI recordings during wakefulness and all stages of NREM sleep. ICA-based DMN mapping, functional connectivity analysis of DMN/ACN nodes, and dynamic tracking in individuals all demonstrated that the DMN can be followed from wakefulness to SWS but that its anatomical configuration undergoes consistent changes.

Demonstration of the DMN during natural human SWS, the deepest sleep stage, approves the robust and autonomous character of the DMN and extend reports on preserved DMN connectivity during light sleep (Horovitz et al. 2008; Larson-Prior et al. 2009) and light sedation (Greicius et al. 2008). It appears that the DMN, when defined as temporally coherent network as a whole, is not bound to wakefulness, preserved

<table>
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<tr>
<th>Table 1</th>
<th>Sleep-stage–dependent contributions to the DMN/ACN</th>
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<tbody>
<tr>
<td>Description</td>
<td>Subregions</td>
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<tr>
<td>Midposterior DMN node</td>
<td>PCC/RspC, (L) precuneus</td>
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<tr>
<td>Midposterior DMN node</td>
<td>PCC and paracentral lobule</td>
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<td>Midanterior DMN node</td>
<td>Medial frontal gyrus</td>
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<td>Parahippocampal area</td>
<td>Parahippocampal gyrus</td>
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<td>Parahippocampal area</td>
<td>Parahippocampal and fusiform gyrus</td>
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<tr>
<td>Inferior temporal area</td>
<td>Inferior and middle temporal gyrus</td>
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<tr>
<td>Temporal lobe</td>
<td>Superior and transversal temporal gyrus, postcentral gyrus, IPL</td>
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<tr>
<td>Temporal lobe</td>
<td>Parahippocampal and fusiform gyrus</td>
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<tr>
<td>Cerebellum</td>
<td>Culmen and cerebellar lingual</td>
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Note: $k$, cluster size in voxels—one voxel covering 8 $\mu$m; L, left; R, right.

Figure 2. Sleep-stage–dependent areas of the DMN/ACN. (A) Clusters represent areas with significant main effect of sleep stage on focal DMN strength ($P_{\text{cluster,FWE}} < 0.05$). Seven out of a total of 8 areas (see Table 1) are depicted. Graph bars represent contrast estimates for each factor level (i.e., sleep stages W, S1, S2, and SWS) as extracted from the peak voxels; horizontal lines indicate significant post hoc comparisons as assessed by $T$ contrasts. Note different dynamics and different final level reached. (B) Highly similar areas as compared with (A) appeared in the comparison of W against sleep (combined S1, S2, and SWS). Note the additional appearance of 2 significant clusters in the posterior ACN nodes, reflecting the increase from negative $Z$ values (anticorrelation) during wakefulness to vanishing contribution during SWS. Graph bars represent average contrast values extracted from the bilateral IPL cluster as defined in wakefulness. Note stable contribution of these nodes to the DMN across the NREM sleep stages.
self-awareness, or conscious mental processing (Buckner et al. 2008; Horovitz et al. 2008). However, we noted changes of the total DMN strength between wakefulness and sleep, changing focal contributions of defined areas to the DMN strength and altered internode synchronization. Thus, the null hypothesis of the DMN not undergoing changes in correlation with physiological vigilance changes can be clearly rejected.

We noted a vigilance correlated decrease of the RspC and PCC contribution to the midposterior DMN node, an area that exhibits the highest metabolic rate in the human brain (Gusnard et al. 2001) and the highest degree of functional connectivity to other brain areas in rs-fMRI BOLD time series (Fransson and Marrelec 2008; Cole et al. 2010; Hayasaka and Laurienti 2010; Tomasi and Volkow 2010). Moreover, this specific area emerged selectively in a post hoc comparison between W and S1 (W > S1, Supplementary Fig. 3), rendering it particularly sensitive to the transition to sleep. This is in contrast to negative reports on light sleep (Horovitz et al. 2008; Larson-Prior et al. 2009) that, however, were limited by small sample sizes. PCC contributions to the DMN were also reported to decrease during light sedation with midazolam (Greicius et al. 2008) and with increased sleep pressure (Sämann et al. 2010). The functional reorganization of this complex during sleep, sedation, and increased sleep pressure is further plausible as different lines of evidence suggest the PCC, precuneal cortex, and RspC to constitute key nodes of the neural network of consciousness (Vogt and Laureys 2005).

Despite the RspC/PCC decreasing its relative contribution to the DMN during sleep, it retained its status as the main node of the DMN that, however, changed its functional connectivity to all other nodes in a sleep-stage-dependent manner.

The mPFC represents the anterior midline node of the DMN. We observed a stepwise decrease of mPFC contribution with eventual fragmentation of the initial coherent cluster. This result goes beyond earlier reports on seed-based DMN extraction during light sleep (Horovitz et al. 2008; Larson-Prior

Figure 3. Result of functional connectivity network analyses of DMN nodes. (A) Binary masks for signal extraction were based on positive and negative T contrast of DMN maps of wakefulness against zero (for details, see Methods). Midline nodes were split into a left and right part. (B) Comparison of average functional connectivity (Fisher’s z’) for each pair of nodes and each sleep stage. Adjusted means as emerging from the mixed model analysis are color coded. P values of the main effect (mixed-model analysis, sleep stage as fixed effect) are given in the mirrored lower left part of the matrix. *P_{Bonf} < 0.05. (C) Color code used for z’ display with bluish values representing negative correlations; legend describes order of sleep stages within each cell (W, S1, S2, SWS).
et al. 2009) and ICA-based DMN analysis during light sedation (Greicius et al. 2008) that showed no mPFC changes. Importantly, the mPFC is known to be integrated in the classical DMN to interindividually different degrees, to the point of divided anterior/posterior DMN (Damoiseaux et al. 2006). We propose that mPFC/posterior node coupling within the DMN is sensitive to vigilance changes and particularly disrupted during SWS. Similarly, Horovitz et al. (2009) observed mPFC/posterior node decoupling in deep sleep as detected by seed-based DMN extraction. Notably, this decoupling is a strong process with drops from $z$-values of about 0.6--0.1, exemplifying that functional connectivity covers a wide dynamic range despite unchanged anatomical connectivity (Skudlarski et al. 2008). The here presented results account only for young, healthy adults, as the strength of the mPFC/posterior node connectivity is also modulated by age as indicated by studies of newborns, children up to 6 years, and older adults (Andrews-Hanna et al. 2007; Fair et al. 2008; Gao et al. 2009). More specifically, reported age-related decreases of DMN functional connectivity were remarkably strong and affected the majority of DMN nodes and the dorsal attention system (Andrews-Hanna et al. 2007). In turn, in the DMN of children at school age was reported as sparsely connected with very low anterior-posterior midline connectivity (Fair et al. 2008). As healthy children and elderly subjects are unimpaired in terms of their general level of vigilance and consciousness, these findings challenge the assumption that functional connectivity and vigilance exhibit a fix coupling across the human age span. Rather, age seems to define a general framework of individual DMN connectivity in which relative sleep-associated changes may occur. As one important determinant of functional connectivity, several studies have highlighted anatomical connectivity that itself is influenced by the myelination status during childhood and adolescence and by white matter degeneration in old age (Andrews-Hanna et al. 2007; Greicius et al. 2009). In this respect, our results corroborate that in addition to age as modulating factor, vigilance should be taken into account in clinical (or normative) DMN studies.

In contrary to the posterior DMN node that remained detectable by the ICA-based approach throughout all sleep stages, the bilateral PHG and right inferior temporal gyrus demonstrated early retraction from the DMN during S1 to the level of no significant contribution during SWS. The PHG DMN compounds were previously reported to be less robustly detectable by the task-induced deactivation approach, and to be less strongly correlated with the midline core nodes of the DMN (Kahn et al. 2008). We add that vigilance may represent a critical determinant for the degree of PHG/hippocampus connectivity to the DMN. The fact that episodic memory reports are more frequent in awakenings arising from sleep onset or light sleep than from SWS (Baylor and Cavallero 2001) is an interesting parallel, evoking the hypothesis that reduced access to episodic memory may represent a behavioral correlate of PHG disintegration. Group and single-case analyses also demonstrated transiently increased temporolateral involvement (mainly BA 22, Fig. 1C, Supplementary Animated Graphics) during S2, that point to complex reorganization of temporal lobe connectivity to the DMN during sleep. Here, further seed-based analysis as employed to dissect functional connectivity of temporomesial structures with both parietal and temporolateral memory systems (Kahn et al. 2008) may be helpful.

Reduced coupling of frontal and parietal neuronal activity during sleep is a phenomenon similarly observed in neurophysiological recordings (Werth et al. 1997; De Gennaro et al. 2001). Furthermore, using focal TMS-induced activation,

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![Figure 4. Distinctness of the DMN from competing components in a single subject through vigilance states. (A) Five of 25 components of the first analysis window (images 1-150, recursive ICA of a single subject) ranked highest in terms of similarity to the DMN template (Supplementary Fig. 2B). Note clear distinctness of the first component compared with the second to fifth component. (B) Pearson correlation coefficients between all 650 x 5 candidate components and the DMN of the first analysis window shown in (A). Data points marked in red represent the stream identified by the similarity-based tracking method (for details, see Methods). Note decreasing similarity of the DMN with the starting DMN, increasing similarity of competing components starting with transition to S1, and partial recovery of the initial pattern in deep sleep.](https://academic.oup.com/cercor/article-abstract/21/9/2082/384811)
Massimini et al. (2005) showed that during sleep propagation of cortical excitation is abolished. Our results showing reduced corticocortical synchronicity with respect to slow spontaneous BOLD fluctuations appear to represent transmodality validation of these EEG and TMS findings: The cortex may be in a sleep-specific state of general inhibited information transmission that also prevents the build-up of interregional synchronicity. Our data add that not only preserved stimulus-induced signal transmission but also spontaneous cortical synchronicity, foremost coupling of the mPFC to the posterior DMN node, is a critical determinant of wakefulness. With fading of consciousness during sleep, this cortical synchronicity retreats. Interestingly, similar DMN/ACN patterns change as in sleep stage 1 can be found after sleep deprivation using analysis of rs-fMRI (Sämann et al. 2010) or task-fMRI with DMN deactivation (Gujar et al. 2010), pointing out that DMN/DAS integrity does not only reflect current vigilance but also aspects of sleep–wake homeostasis.

Our results do not suggest that mental processing is annihilated during sleep. In fact, reports of NREM mentation showed that dream-like experiences may be present throughout all sleep stages, though to a reduced extent in deep NREM sleep as compared with light NREM or REM sleep (Nielsen 2000; Tononi 2009). Notably, NREM mentations tend to be very short and rather thought like (Tononi 2009), suggesting that embedding of the mPFC in the DMN plays a critical role for the integration of sleep mentations.

The existence of areas with temporal anticorrelation to the DMN has been reported before (Fox et al. 2005; Fransson 2005, 2006; Uddin et al. 2008) and could be reproduced in our analysis (Figs 1A,B and 3A). The ACN detected by ICA in wakefulness comprised the bilateral IPL and insula that are generally assigned to the ventral attention system (Fox et al. 2006). The right insula/prefrontal and the IPL node of the ACN, however, matched previously reported areas of overlap between the ventral and dorsal attention (Fox et al. 2006), suggesting that the ACN of this analysis comprised nodes of both attention networks. Using ICA, we found that the anticorrelation between the ACN and the DMN as a whole diminished to the level of no significant contribution during S2 and SWS. CCA differentiated that in wakefulness the bilateral insular nodes of the ACN were more strongly connected to the PCC/RspC, whereas the IPL/ACN nodes were more strongly connected to the mPFC that is in agreement with reported anticorrelation patterns of the DMN in independent samples (Uddin et al. 2008; Sämann et al. 2010). Further, the preferentially connected pairs (PCC/RspC-insula and mPFC-IPL/ACN) were less affected by sleep than connections across these 2 subsystems, for example mPFC-insula. Within the ACN, about half of the connections showed marginally significant decreases in connectivity and half showed stable connectivity (Fig. 3R, Supplementary Table 3a–f), indicating that the nodes primarily defined by their anticorrelation properties in wakefulness still represent a coherent network in sleep. Larson-Prior et al. (2009) reported slightly increased autocorrelation among nodes of the dorsal attention system during light sleep; however, due to the extraction of this network by a seed technique, findings are not directly comparable. Our results point mainly to partially decreased coupling between the DMN and ACN, and to largely preserved connectivity among nodes of the ACN. Boly et al. (2007) reported that during wakefulness, higher activity of the dorsal attention system predicted conscious stimulus perception. We speculate that the combination of DMN/ACN decoupling and ACN disintegration on the one hand and partial preservation of DMN/ACN coupling on the other hand could account for the reduced, yet not completely extinguished responsiveness to external stimuli during sleep.

While the PCC/RspC and mPFC exhibited reduced integration in the DMN during deepening of sleep, the differential contrast of DMN maps did not reveal a sleep-stage–dependent effect in the lateral parietal nodes of the DMN. CCA added that also the connectivity between these nodes was among the most stable connections. In contrast, in sleep, subareas of the posterior medial node were "unlinked" from the DMN. As for CCA, the regions of interest were defined from the entire nodes as detected in wakefulness; this subregional change within the PCC/RspC may well explain decreased connectivity between the PCC/RspC and IPL/DMN mainly observable in light NREM sleep. Interestingly, this connectivity was restored to levels not different from wakefulness in SWS (Fig. 3A, Supplementary Table 3e). This latter observation as well as increasing connectivity between the left and right PCC/RspC, and remarkably stable connectivity within the mPFC, are in line with previously reported results of a comparison between wakefulness and deep sleep (Horovitz et al. 2009). Overall, these exemplary observations on the posterior areas of the DMN emphasize that vigilance is not related to disintegration of the DMN in a linear fashion. Rather, the parallel occurrence of reduced integration of some subareas of the DMN (e.g., PCC/RspC and PHG) and stability of other (sub)nodes suggests that sleep interacts with the DMN in a subregionally differential manner. This line of interpretation is supported by recent results on the functional architecture of the DMN during wakefulness as investigated by hierarchical clustering analysis (Andrews-Hanna et al. 2010): this analysis segregated the DMN into a midline core (anterior mPFC and PCC) and 2 distinct subsystems, the medial temporal lobe subsystem (RspC, PHG, posterior IPL, ventral mPFC) and the dorsomedial PFC subsystem (temporoparietal junction, TL, temporal pole, dorsomedial PFC). Yet, further hypothesis-driven analyses are needed to clarify the role of these demarcations in sleep and the physiological role of resting state (sub)networks that are maintained in sleep.

In this study, we employed both ICA and CCA as complementary techniques to analyze functional connectivity (Ma et al. 2007; Auer 2008). ICA decomposes the data into anatomical modules with maximal spatial and temporal dissimilarity. It is particularly useful to separate out BOLD fluctuations caused by motion, respiration, CSF and venous pulsations, other vascular phenomena, or susceptibility artifacts, and eventually identify components that reliably represent neural sources (Damoiseaux et al. 2006; Ma et al. 2007; Birn et al. 2008). Regional network changes can then be explored using voxelwise statistics (Sorg et al. 2007; Greicius et al. 2007, 2008) whereby component selection is a critical step. To exclude that sleep-induced DMN changes may have influenced the component selection process, we also analyzed the second to fifth best frequency/template matches (Supplementary Fig. 2), finding no systematic sleep-stage–dependent shift toward a "split DMN" and no sleep-stage–dependent differential effects of across the second to fifth best DMN matches. Another advantage of ICA-based network analysis is its explorative character, allowing to detect nonhypothesized
inclusions of areas into a network, as for example reported for major depression (Greicius et al. 2007). For the analysis of functional connectivity within a hypothesized network, CCA based on clearly defined bivariate correlations is useful (Ma et al. 2007). The characteristics of extracted time courses, however, are dependent on spatial and temporal preprocessing and definition of seeds (Ma et al. 2007; Weissenbacher et al. 2009). This type of analysis, however, is desensitized by any unspecific contribution from the white matter or CSF compartment or motion-correlated signal. In order to direct the analysis to hypothesized DMN/ACN changes, we selected core regions of this network with maximum overlap between subjects, defined during wakefulness. Further, time courses were extracted from residual images cleaned from motion effects, global white matter, and CSF signal as methodologically validated (Weissenbacher et al. 2009). Both approaches converged in that lower vigilance was associated with a weaker functional connectivity between its major nodes.

Recursive ICA and seed correlation applied to an individual in a sliding window technique allowed for continuous monitoring of DMN development during sleep onset (Supplementary Animated Graphics). Both tracking methods revealed a similar development of the DMN through the sleep stages as the group analysis and exemplified the true degree of a similarity measure between subsequent components in time rather than the difference to a static template. Still, the resulting stream contains some instability of the posterior midline node (see Supplementary Animated Graphics) that might be related to this area being represented in a second DMN component. Other analysis methods such as graph theory analysis might help to investigate aspects of connectivity across networks including changes of cortical modularity and "small worldness" in more detail.

One limitation of this study is its restriction to the DMN and ACN. Other sensory and cognitive networks can consistently be found during wakeful resting (Damoiseaux et al. 2006) and in light sleep (Larson-Prior et al. 2009). The present analysis focused on the DMN and ACN because of their relation to external and internal awareness that obviously change during sleep. Complementary studies using group ICA methods or cross-correlation analyses (CCAs) are needed to complete the picture of resting functional connectivity changes in sleep and to study the question if sleep-specific processes related to synaptic plasticity and long-term memory consolidation (Buszaki and Draguhn 2004) are reflected in resting networks unique to the sleeping state.

In summary, using voxelwise ICA-based group analysis, dynamic tracking of the DMN in individual subjects, and CCA, we detected activity of the DMN throughout all human NREM sleep stages. Vigilance was found to be a critical determinant of overall and of focal DMN strength. Decoupling of defined areas of the DMN was seen in dependency of the sleep stage. Here, temporomesial areas and the anticorrelated DAS decreased their contribution to nonsignificant levels whereas the midposterior and midanterior nodes remained active at lower levels. Particularly, the reorganization of the mPFC and temporomesial areas and the retraction of the ACN may represent functional substrates of sleep-related phenomena, such as loss of consciousness, loss of memory access, and reduced external awareness that should be object of future studies.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

Notes
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References