

# AN EEG-fMRI STUDY OF THE DEFAULT NETWORK IN LIGHT SLEEP

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## INTRODUCTION

The advent of techniques that allow the simultaneous acquisition of fMRI and EEG enable investigation of global brain activity across states of consciousness (described as subjective perception of the external world). While many studies of functional brain activity have identified dynamic network interactions among brain regions, recent investigations of low-frequency fluctuations in the fMRI-BOLD signal have identified a limited set of regional networks that appear to fluctuate coherently even in situations in which those regions are not actively engaged in an experimentally-imposed task. There is great interest in better characterizing these networks, particularly the "default" or "task negative" network which is made up of brain regions showing the greatest activity during quiet, non-goal directed, rest. One difficulty is determining whether the unconstrained nature of a "non-task" allows for covert and therefore ill-defined attentional states that could contaminate interpretation of coherent activity. One approach to this problem is to examine the behavior of the default network when the brain is disengaged from the external environment. This condition is met in non-rapid eye movement (NREM) sleep. Accordingly, we investigated the functional connectivity of the default system in awake and early NREM sleep states using simultaneously acquired fMRI-BOLD and EEG. Analyses also included the dorsal attentional network (Corbetta et al., 2002) with which the default system has been reported to be anti-correlated (Fox et al., 2005).

## METHODS

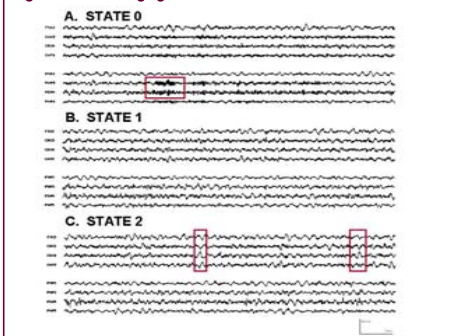
**Subjects.** Ten right-handed, healthy human subjects (ages 22-54, 5 females; Table 1) were recruited from the campus of Washington University under a protocol approved by the University's Human Studies Committee. All subjects gave informed consent and were reimbursed for their participation. Two subjects returned for a second sleep study.

**Data Collection.** Whole brain fMRI (Siemens Allegra 3T scanner; TE = 30 ms, 4 mm<sup>3</sup> voxels, 2.013 sec/volume, 1 sec gap) was acquired in the eyes closed resting state using a signal/noise enhanced EPI sequence. Runs were 20-60 minutes in duration in 20 minute (398 volumes) segments. Gradients continued to run in the 45 sec pause between segments, which was used to refresh the EEG acquisition without disturbing the subject. Preprocessing of the BOLD timeseries included head motion correction within and across segments and atlas transformation on the basis of T1- and T2- weighted structural images. EEG data were simultaneously acquired (DC-3500 Hz, 20 KHz sampling rate) using the MagLink™ (Compumedics Neuroscan, TX) system (modified 10/20, 64 electrodes) and the Synamps/2™ amplifier. Gradient artifact was reduced using Scan 4.5 software. Ballistocardiogram artifact was reduced using in-house software (Vincent et al., 2005).

**Analysis.** Following preprocessing (Fox et al., 2005), functional connectivity was assessed by extracting the averaged BOLD time series from 12 mm-diameter-sphere volumes centered on the foci defined in Table 2. The extracted seed time-series was then correlated to all other brain voxels to produce spatial correlation maps. We also computed the correlation matrix of all seed and system regions. This matrix was used to evaluate correlations within and between identified networks during quiet waking and asleep. Seeds defined (Table 2) for the task-positive attention network (Fox et al., 2005) were centered on the intraparietal sulcus (IPS), the middle temporal region (MT) and the frontal eye field (FEF) region; for the task negative (Fox et al., 2005) or default (Raichle et al., 2001) system, seeds were centered on the medial prefrontal cortex (MPF), the lateral parietal cortex (LP) and the posterior cingulate/precuneus region (PCC). Results are displayed as Fisher-z transformed correlation values.

**Statistical analysis.** Random effects analyses were performed on group spatial map data and corrected for multiple comparisons at the p = 0.01 level. Fisher-z transformed correlation values were averaged across subject by state and evaluated for statistical significance using JMP5.1.

Figure 1: EEG Staging



Subjects' EEG was staged in 30 second epochs according to the following criteria:  
**State 0:** An awake EEG showing posterior alpha activity (red box) with eyes closed  
**State 1:** Drowsy-stage 1 sleep showing a general slowing of the EEG and a loss of the alpha rhythm that marked stage 0  
**State 2:** Stage 2 sleep showing increased slowing of the EEG and bursts of vertex dominated activity (lower boxes) and is probably equivalent to stage 2 sleep.  
 States 0 and 1 were grouped into stage wake while state 2 was considered stage 2 NREM sleep.

MI. TABLE 1: Subject demographics, and the amount of time spent in defined sleep stage

TABLE 1: SUBJECT INFORMATION					
SUBJECT	AGE	ST0	ST1	ST2	
18524 <sup>†</sup>	27	3.9 min 77 frames	11.3 min 226 frames	24.8 min 493 frames	
19983 <sup>*</sup>	54	14 min 279 frames	18.9 min 376 frames	26.5 min 527 frames	
20446	26	12.6 min 251 frames	13.9 min 278 frames	33.4 min 685 frames	
20608	26	M	M	M	
21203 <sup>†</sup>	27	3.7 min 71 frames	16.4 min 327 frames		
21236	30	18.6 min 379 frames	20.6 min 411 frames		
21363	25	51.3 min 1022 frames	8 min 160 frames		
21506	27	20min 396 frames			
21672	23	7.8 min 156 frames	1.9 min 38 frames	29.5 min 588 frames	
21950	22	60 min 1194 frames			
22062	27	7.5 min 150 frames	15.4 min 306 frames	14.7 min 292 frames	
22098 <sup>*</sup>	54	48.8 min 972 frames			

<sup>††</sup> Replicate subjects, M=excessive movement precluding scoring

MII. TABLE 2. The seed regions used in this study are defined below.

TABLE 2: REGIONAL NETWORK SEEDS				
NETWORK	SEED REGION NAME	TALAIRACH COORDINATES		
		X	Y	Z
DEFAULT	Left Posterior Parietal/Precuneus (PCC)	-2	-36	37
	Left Medial Prefrontal (LMPF)	-3	39	-2
	Right Medial Prefrontal (RMPF)	1	54	21
	Left Lateral Parietal (LLP)	-47	-67	36
	Right Lateral Parietal (RLP)	53	-67	36
ATTENTION	Left Intraparietal Sulcus (LIPS)	-23	-66	46
	Right Intraparietal Sulcus (RIPS)	25	-68	52
	Left Frontal Eye Fields (LFEF)	04	-12	61
	Right Frontal Eye Fields (RFEF)	28	-7	54
	Left Middle Temporal (LMT)	-47	48	-3
	Right Middle Temporal (RMT)	54	-43	8

Seeds derived from Fox et al (2005)

## RESULTS

Ten subjects participated in this study which included 12 experimental nights (all scans were performed between 5-10 PM). Of these, as noted in Table 1, one dataset was removed from analysis due to excessive motion and 4 subjects were unable to sleep. Data is reported for 5 subjects who reached stable non-rapid eye movement (NREM) sleep in six experimental session.

EEG data were scored as illustrated for one subject in Figure 1. States 0 and 1, which are analogous to sleep stages wake and 1 were combined into Stage Wake, while state 2, which represented early NREM sleep, constituted Stage Sleep.

Figure 2: Seed Regions of Interest

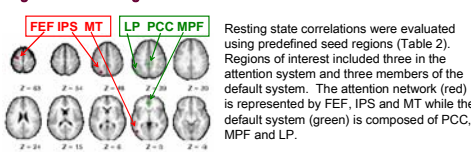


Figure 3: System connectivity evaluated using distributed system region-of-interest

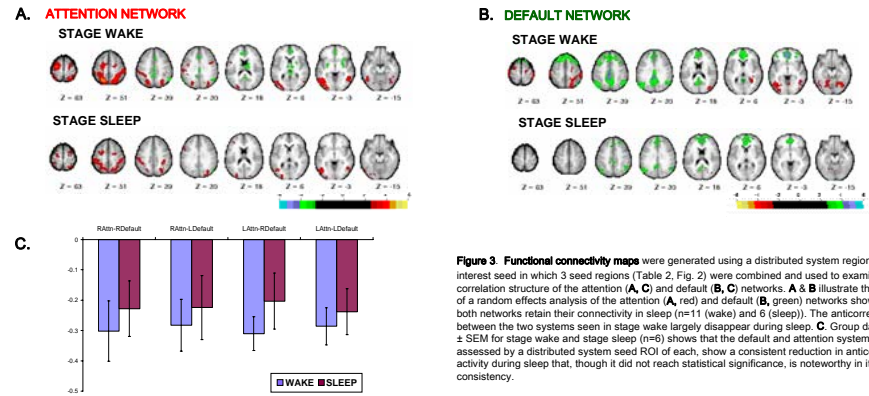


Figure 3. Functional connectivity maps were generated using a distributed system regions of interest seed in which 3 seed regions (Table 2, Fig. 2) were combined and used to examine the correlation structure of the attention (A, C) and default (B, C) networks. A & B illustrate the results of a random effects analysis of the attention (A, red) and default (B, green) networks showing that both networks retain their connectivity in sleep (n=11 (wake) and 6 (sleep)). The anticorrelations between the two systems seen in stage wake largely disappear during sleep. C. Group data (mean ± SEM for stage wake and stage sleep (n=6)) shows that the default and attention systems, assessed by a distributed system seed ROI of each, show a consistent reduction in anticorrelated activity during sleep that, though it did not reach statistical significance, is noteworthy in its consistency.

Figure 4: System connectivity evaluated using single seed region-of-interest

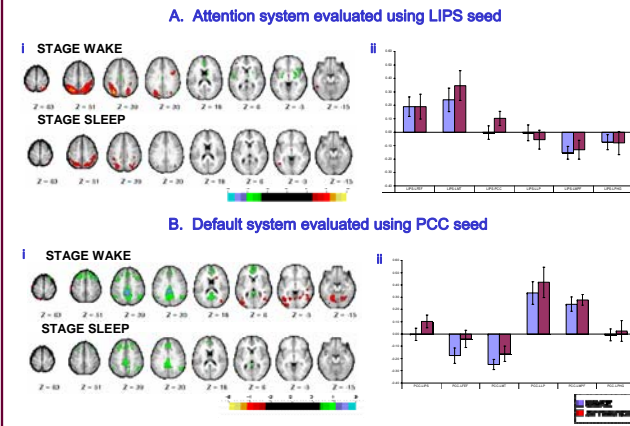


Figure 4. Functional connectivity maps were generated using a single seed regions-of-interest for each system. This strategy allowed a closer examination of correlated activity within each defined system, and is the strategy used in other seed based functional connectivity studies. In (A), the LIPS was chosen as a representative seed for the attention system, while in (B) the PCC was chosen as representative of the default system. In Aii and Bii histograms show the correlation values for the seed region reference compared to seeds comprising attention (LFEF, LMT) and default (LLP, LMPF, LPHG) systems. Seed region referents are not compared to themselves. Note that (1) the anti-correlations previously reported to exist between default and attention systems remain regardless of which seed was chosen or whether the subjects were awake or asleep, (2) that there was an overall trend for no change, or a slight increase, in the strength of within-system correlations with sleep, and (3) that between systems, anticorrelations were reduced in sleep relative to wake.

## DISCUSSION

Resting state functional connectivity has attracted a great deal of recent interest (Raichle et al., 2001; Greicius and Menon, 2004; Larson-Prior et al., 2005; Fox et al., 2005; Beckman et al., 2005; Damoiseaux et al., 2006) as it suggests that a limited set of regions remain connected as a network even if their activation isn't being evoked by experimentally defined tasks. Among the networks so defined are the default network, first defined on the basis of its reduced blood flow during cognitive task performance (Shulman et al., 1997) and an attention network comprised of components defined by Corbetta and colleagues as a dorsal frontoparietal system functioning in the top-down, cognition-driven control of attention (Corbetta & Shulman, 2001; Hahn et al., 2006). Using a subset of the regions described as components of these two systems, Fox and colleagues (Fox et al., 2005) demonstrated anti-correlated spontaneous activity between them, lending credence to earlier hypotheses that the default system acted in inwardly focused, self-referential modes which were increased as the demand for attention to the external world was reduced. Importantly, both PET (Maquet, 2000) and fMRI (Kaufman et al., 2006) studies have demonstrated decreased activity in components of both of these networks in NREM sleep, with PCC showing significant deactivation not only in sleep but also in states of reduced consciousness such as anesthesia and persistent vegetative states. An evaluation of the functional connectivity of these important systems in sleep, where the brain is increasingly disconnected from the external world, is of clear import. We report here that functional connections are preserved in these networks as consciousness is reduced with the descent into sleep. Evaluated both as single seed and distributed system ROIs, both attention and default systems maintained their functional connectivity in NREM sleep although measures of global activity in components of both systems (notably PCC, PHG in default and IPS and frontoparietal lobes in attention systems) show strong decreases in this state. The clearest change was the reduction in the anticorrelated activity of these two networks in NREM sleep.

## ACKNOWLEDGEMENTS

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## REFERENCES

- Beckman, C.F., DeLuca, M., Devlin, J.T. et al. *Phil Trans. R. Soc. B* (2005): 360:1001
- Corbetta, M. and Shulman, G. *Nat. Rev. Neurosci.* (2002) 3:210
- Damoiseaux, J.S., Rombouts, S.A.R.B., Barkhof, F. et al. *PNAS* (2006): 103:13848-13853
- Fox, M.D., Snyder, A.Z., Vincent, J.L., et al. *PNAS* (2005): 102:9673-9678
- Greicius, M.D. and Menon, V. *J. Cog. Neuro.* (2004): 16:1484
- Hahn, B., Ross, T.J., Stein, E.A., *Neuroimage* (2006) 32:842
- Kaufman, C., Wehrle, R., Wetter, T.C. et al. *Brain* (2006) 129:655
- Larson-Prior, L.J., Zempel, J., Prior, F.W. et al. *Neuroimage* (supp. 1) (2006) 31:187
- Maquet, P. *J. Sleep Res.* (2000): 9:207
- Raichle, M.E., MacLeod MA, Snyder AZ, et al. *PNAS* (2001) 98:676-682
- Shulman, G.L., Fiez, J.A., Corbetta, M. et al. *J. Cog. Neuro.* (1997) 9:648
- Vincent, J.L., Zempel, J., Larson-Prior, L.J., et al. *SFN Abstract* (2005) 450.20