Larson-Prior, L.J.*; Zempel, J.²; Vincent, J. L.¹; Nolan, T.S.¹; Snyder, A. Z.¹,²; Raichle, M. E.¹,²
Depts. of Radiology and Neurology², Washington University in St. Louis, MO, USA

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 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-attended, rest. One difficulty is determining whether the performance correlation analysis is a “true” allochrome for covert and therefore implicit 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 voxels, 2.01236 image; 1 sec gap) was acquired in the eyes closed resting state using a signal-enhanced EPI sequence. Runs were 20-60 minutes in duration in 20 minute (395 volumes) segments. Gradients continued to run in the 45 sec between segments, which was used to reflush the EEG acquisition without disturbing the subject. Preprocessing of the BOLD time series 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 Neurac, TX) system (modality 1020, 64 electrodes) and the DynaComp™ amplifiers. Gradiometer artifact was reduced using Scan 4.5 software. Ballistocardiogram artifact was reduced in-house software (Vincent et al., 2005).

Analysis: Following preprocessing (Fox et al., 2005), functional connectivity was assessed by examining 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 regions to produce spatial correlation maps. We also computed the correlation maps of all seed and system regions. The matrix was used to evaluate correlations within and between identified networks during quiet waking and sleep. Seeds defined (Table 2) for the task positive attention (Fox et al., 2005) network were centered on the intraparietal sulcus (IPS), the middle temporal region (MT) and the frontal eye field (FEF) for the task negative network (Fox et al., 2005) or default (Raichle et al., 2001) system, as well as consistent with the lateral prefrontal cortex (LPC), the lateral parietal cortex (LP) and the posterior cingulate/pinealocerebellum region (PCC). Results are displayed as z-score transformed correlation values.

Statistical analysis: Random effects performed on group spatial 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.

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 (NREMS) sleep in six experimental sessions.

EEG data were scored as illustrated 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 2NREM sleep. Data from the left parietal electrode 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 (NREMS) sleep in six experimental sessions.

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DISCUSSION

State functional connectivity has attracted a great deal of recent interest (Raichle et al., 2001; Greicius and Marron, 2004; Larson-Prior et al., 2005; Fox et al., 2005; Bezman et al., 2005; Damoiseaux et al., 2006) as it suggests that a limited set of regions remain connected even if their activation is not 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 frontal system functioning in the top-down, cognition-driven control of attention (Corbetta & Shulman, 2001; Hahn et al., 2008). 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 (Raichle) (2001) demonstrated decreased activity in components of both these networks in NREM sleep, with PET 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 networks (notably MCC, PHG in default and IPS and frontopolar lobes in attention systems) were attenuated in this stage. The change of interest was the reduction in the anticorrelated activity of these two networks in NREM sleep.

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