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fMRI differences between early and late stage-1 sleep

Dante Picchioni[a,*], Masaki Fukunaga[b], Walter S. Carr[c], Allen R. Braun[d], Thomas J. Balkin[a], Jeff H. Duyn[b], Silvina G. Horovitz[b]

[a] Division of Psychiatry and Neuroscience, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910, USA
[b] National Institute of Neurological Disorders and Stroke, National Institutes of Health, USA
[c] Combat Casualty Care/Trauma & Resuscitative Medicine, Naval Medical Research Center, USA
[d] National Institute on Deafness and Other Communication Disorders, National Institutes of Health, USA

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ABSTRACT

This study sought to test for differences in regional brain activity between stage-1 sleep immediately following wake and immediately preceding stage-2 sleep. Data were collected during daytime fMRI sessions with simultaneous EEG acquisition. A stage-1 interval was defined as follows: ≥30 s of wake, immediately followed by ≥60 s of continuous stage 1, immediately followed by ≥30 s of stage 2. We compared brain activity between the first 30 s of stage 1 (early stage 1), the last 30 s of stage 1 (late stage 1), and isolated wake. A conjunction analysis sorted each voxel into one of a series of mutually exclusive categories that represented the various possible combinations of a significant increase, decrease, or no difference among these three states. The initial dataset consisted of 14 healthy volunteers. A total of 22 sessions in these participants yielded six stage-1 intervals (from four participants) that met criteria for inclusion in the analysis. There were multiple clusters of significant voxels. Examples include changes in default-mode network areas where activity increased compared to wake only in early stage 1 and a bilateral change in the hippocampus where activity increased compared to wake only in late stage 1. These results suggest that activity in anatomically identifiable, volumetric brain regions exhibit differences during stage-1 sleep that would not have been detected with the EEG. These differences may also have specific relevance to understanding the process of sleep onset as well as the neural mechanisms of performance lapses during sleep deprivation.

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The current scoring system for human sleep [13] advanced sleep research and medicine by providing a standard for comparing sleep studies conducted at different times and laboratories. However, this system uses visual scoring of the electroencephalogram (EEG). The EEG cannot easily resolve anatomy in three-dimensional space and the scoring collapses sleep into a series of discrete stages that are not based on any inherent neurophysiological processes that might correlate with waking outcomes. Revisions of this system [10] essentially suffer from the same limitations. As a result, it is likely that anatomically distinct and functionally important sleep processes have been overlooked or even obscured.

Previous studies that sought to address this problem while continuing to use the EEG are important because they compared brain activity within the traditional sleep stages [e.g., 8]; however, the continued use of solely the EEG precludes obtaining information regarding activity in volumetric brain regions. While there are approaches that – in the context of certain assumptions – potentially allow one to suspend the inverse problem and obtain spatial information from the EEG, it is not inherently a technique that provides such information whereas this is exactly the case for functional neuroimaging techniques. Previous studies that sought to address this problem using positron emission tomography or functional magnetic resonance imaging (fMRI) are important because they provide information regarding activity in volumetric brain regions [e.g., 3]; however, these studies only compared brain activity between the traditional sleep stages. An approach that simultaneously examines changes in regional brain activity within the boundaries of sleep stages could serve as a proof-of-concept that functional imaging techniques can provide more information than the EEG. This information may ultimately lead to an increased understanding of why it has proven difficult to predict waking outcome of sleep abnormalities [7,12].

The present study adopted this approach in the context of sleep onset and compared fMRI activity between stage-1 sleep immediately following wake and immediately preceding stage 2. The purpose was to address whether fMRI can indeed identify any dif-

* Corresponding author. Tel.: +1 301 319 3106; fax: +1 301 319 9979.
E-mail address: dante.picchioni@amedd.army.mil (D. Picchioni).

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ferences within a sleep stage and begin to assess what implications these differences would have for understanding the process of sleep onset.

Data for this analysis were obtained as part of a larger study that assessed whether default-mode network activity continued during light sleep [9]. The study protocol was approved by the National Institute of Neurological Disorders and Stroke Institutional Review Board; all participants gave written informed consent.

Data were collected in 22 sessions from 14 participants. From this sample, six stage-1 intervals (see definition below) were identified from four participants (two males; ages 23, 28, 38, 38). All participants were free of neurological disorders and reported normal sleep/wake patterns.

Neither sleep deprivation nor any other experimental manipulation was used to facilitate sleep in the scanner. All sessions occurred during the normal workday and lasted 60 min. For a period of 48 min, the participants were instructed to close their eyes, relax, and try to sleep. During the remaining 12 min, subjects were asked to remain awake while performing a simple visual task (excluded from the present analysis).

EEG acquisition hardware included silver–silver chloride sintered electrode caps with carbon fibers (MagLink) and MRI-compatible amplifiers (Synamps2); data acquisition and processing was performed using Scan 4.3 software (all from Neuroscan, Compumedics USA Ltd., El Paso, TX). Forty electrodes, including those in the standard 10–20 International system, were used. The ground electrode was located anterior to Fz and the reference electrode was between Cz and Pz. Two bipolar electrodes were used to monitor electro-oculogram as well as the cardiac signal. Acquisition rate was 10 kHz, with a low-pass filter set to 3 kHz.

After low-pass filtering at 250 Hz, artifacts induced by MRI gradient switching were removed based on template subtraction [1]. Subsequently, bandpass filtering was performed using a frequency range of 0.5–28.0 Hz. Cardio-ballistic artifact was removed by detecting the R-wave, creating an averaged template of the artifact, extracting its principle components (using principle component analysis), and removing those components from the original data.

Sleep was scored by a Diplomate of the American Board of Sleep Medicine (TJB) according to standard criteria [13]. Given the data collection period took place during the day and did not incorporate any prior experimental sleep deprivation, the participants rarely obtained any sleep deeper than stage 1. This was viewed as an opportunity to examine stage-1 sleep because it is generally considered to be a transitional stage and could potentially represent sleep onset processes. The scored records for all the initial participants were reviewed for intervals of stage-1 sleep that met the following criteria: $\geq$30 s of wake, immediately followed by $\geq$60 s of continuous stage 1, immediately followed by $\geq$30 s of stage 2. The basic form of the analysis consisted of contrasting the brain activity among the first 30 s of stage 1 (early stage 1 or E1), the last 30 s of stage 1 (late stage 1 or L1), and isolated wake (W). Isolated wake was defined as an epoch of scored wakefulness that was separated from epochs of sleep by $\geq$30 s. A total of six stage-1 intervals (including six intervals of E1 and L1 each) were identified from four participants, which resulted in 30 fMRI volumes (time points) of E1 and L1 each. These four participants contained 840 fMRI volumes of W (225, 245, 185, and 185, respectively).

Blood oxygen level dependence (BOLD) fMRI was acquired on a 3 T scanner (GE Signa, Milwaukee, WI, USA) using a 16-channel receive-only detector array head coil (Nova Medical, Wakefield, MA) [4]. Single-shot echo-planar images were collected from 28 oblique-axial slices (1.7 mm $\times$ 1.7 mm nominal in-plane resolution, 3.0 mm thickness, 0.5 mm gap) covering most of the brain. The 16-channel coil employs a helmet-type construction that minimizes the distance between the individual coils in the array and the scalp, but results in somewhat poor coverage of the cerebellum and lower brain stem.

The excitation flip angle was 90°. Repetition time was 6 s and echo time was 43 ms. 3D T1-weighted MPRAGE images were collected from all volunteers using a standard GE head coil in a separate session. Respiration was measured using respiratory bellows and cardiac rate was measured using a pulse oximeter. These signals were collected using custom software at a frequency of 1 kHz.

fMRI analysis was performed using IDL 6.2 (ITT visual information solutions, Boulder, CO, USA), SPM2 (Wellcome Department of Cognitive Neurology, London, UK), and AFNI (National Institutes of Mental Health, Bethesda, MD, USA). IDL and SPM2 were used for pre-processing the data while AFNI was used for additional pre-processing and for the statistical analyses. Processing prior to statistical modeling included slice timing correction, rigid body motion correction, baseline drift removal using a high-pass frequency filter (0.006 Hz), global signal correction using the within-volume mean as a linear regression covariate, a 4 mm Gaussian blur, masking functional activity outside the brain, transformation to percent signal change using the within-volume mean, and warping the structural and functional images to Talairach space.

The cardiac rate and the respiration volume per unit time – computed as the peak to peak amplitude of the respiratory trace [2] – were also entered as linear regression covariates. The cardiac rate and respiration covariates were estimated by correlating the physiological signals with the fMRI BOLD signal for each voxel at different delays (up to $\pm$10 TRs), averaging the correlation coefficient across the whole brain for each delay, and selecting the delay with highest correlation (typically 1 or 2 TRs).

The fMRI analysis was a block design where the blocks were associated with the polysomnographically defined sleep stages described above. The categorical variables that represented E1, L1, and W were dummy coded into two dichotomous variables with wake as the reference level, and convolved with a canonical hemodynamic response function.

The analysis then proceeded according to a standard two-level approach at the individual participant and group level. At the individual participant level, the predicted hemodynamic activity was correlated with the observed hemodynamic responses to yield separate regression coefficients for E1 and L1. At the group level, these coefficients were entered into a two-way mixed-model factorial analysis of variance with participants as a random factor and E1 or

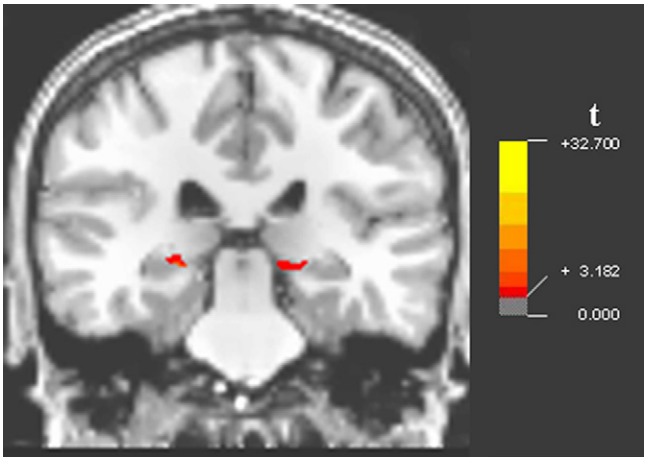

Fig. 1. Example image from one of the significant clusters (hippocampus) from one of the conjunction categories (Unique L1 Increases: E1 = W, L1 > W, L1 > E1).

Table 1
Clusters of significant voxels for each conjunction category

	Left hemisphere							Right hemisphere						
	Voxels	E1 vs W |t|	L1 vs W |t|	L1 vs E1 |t|	x	y	z	Voxels	E1 vs W |t|	L1 vs W |t|	L1 vs E1 |t|	x	y	z
Unique L1 increases														
Hippocampus	270	ns	4.2	32.7	−26	−23	1	162	ns	3.5	13.4	19	−33	0
Precentral G – 4/6	14	ns	3.5	5.1	−47	−8	46	25	ns	3.5	5.1	61	−4	38
Angular gyrus – 39/occipital – 19	–	–	–	–	–	–	–	54	ns	4.2	7.2	39	−71	34
Inf frontal G – 44/pars opercularis	–	–	–	–	–	–	–	76	ns	4.0	4.1	32	16	27
Middle frontal G – 6/8	–	–	–	–	–	–	–	315	ns	4.9	18.0	28	12	44
Medial prefrontal gyrus – 8	–	–	–	–	–	–	–	95	ns	4.9	5.0	12	24	46
Unique L1 decreases														
Inf parietal sulcus – 40/7	126	ns	3.5	5.1	−31	−52	43	–	–	–	–	–	–	–
Middle frontal G/9	172	ns	4.6	29.7	−35	8	36	–	–	–	–	–	–	–
Precentral G/6	25	ns	3.4	9.7	−37	4	41	–	–	–	–	–	–	–
Sup parietal lobule/precuneus – 7	90	ns	3.2	12.4	−14	−59	45	–	–	–	–	–	–	–
Sup Frontal G – 6	–	–	–	–	–	–	–	50	ns	4.0	6.5	14	10	51
Unique E1 increases														
Inf parietal lobule/sulcus – 40/7	63	3.5	ns	32.7	−25	−50	42	254	6.8	ns	32.7	36	−49	42
Middle frontal G – 9/6	127	3.3	ns	11.1	−36	6	41	–	–	–	–	–	–	–
Inf parietal sulcus/precuneus – 7	288	4.0	ns	29.4	−24	−42	45	–	–	–	–	–	–	–
Precuneus – 7	134	3.2	ns	4.3	−6	−59	57	–	–	–	–	–	–	–
Precuneus – 7	380	6.6	ns	6.4	−19	−62	41	–	–	–	–	–	–	–
Sup frontal G – 6	–	–	–	–	–	–	–	181	4.3	ns	7.4	15	11	50
Sup parietal lobule – 7	–	–	–	–	–	–	–	74	3.3	ns	4.4	17	−57	60
Unique E1 decreases														
Precentral G – 4/6	332	4.0	ns	12.1	−45	−5	50	358	3.6	ns	10.3	61	−10	40
Middle frontal G/9	–	–	–	–	–	–	–	549	3.2	ns	9.6	31	24	25
Ventral thalamus	–	–	–	–	–	–	–	56	4.0	ns	7.7	11	−20	0
Early (▼), late (▲)														
Precentral G – 6	29	6.6	7.5	7.5	−46	−6	49	–	–	–	–	–	–	–
Middle frontal G – 8	–	–	–	–	–	–	–	25	3.3	3.4	7.0	25	19	35
Middle frontal G – 9	–	–	–	–	–	–	–	75	7.0	3.4	6.1	31	19	25
Early (▲), late (▼)														
Middle frontal G – 9	45	4.5	4.1	26.9	−36	10	38	–	–	–	–	–	–	–
Precuneus – 7	53	3.8	3.4	25.3	−19	−62	40	–	–	–	–	–	–	–

Numbers following anatomical labels are Brodmann areas; *t* values (absolute values) are the values associated with each particular pairwise contrast; *x*, *y*, and *z* are Talairach coordinates in L–P–I orientation. *Abbreviations*: E1 = early stage 1, L1 = late stage 1, W = isolated wake, Inf = inferior, Sup = superior, G = gyrus; conjunction categories: unique L1 increases (E1 = W, L1 > W, L1 > E1), unique L1 decreases (E1 = W, L1 < W, L1 < E1), unique E1 increases (E1 > W, L1 = W, L1 < E1), unique E1 increases (E1 > W, L1 = W, L1 < E1), early (▼), late (▲) (E1 < W, L1 > W, L1 > E1), early (▲), late (▼) (E1 > W, L1 < W, L1 < E1).

L1 as a fixed factor. Finally, the *t* values associated with each of the three pairwise contrasts among E1, L1, and W were exported into three separate three-dimensional datasets.

A conjunction analysis was performed using the results from these contrasts. This analysis sorted every voxel in the brain into one of 10 categories where W was considered the baseline. It was critical to use a baseline and compare the activity of the two sleep states with it given the relative nature of the fMRI signal [6]. In this context, wake represents a near optimal baseline just as in task-dependent studies, rest is used as a near optimal baseline. The categories represent the mutually exclusive and logically exhaustive combinations of significant differences ($p < 0.05$) or lack thereof among the three pairwise contrasts whenever there was a difference between E1 and L1. In other words, the probability values given as output from the second-level analysis were compared using Boolean logical operators so all possible combinations of results were represented. This created a series of binary datasets that, in total, classified each voxel by fulfilling the logical operators associated with that category. These dataset for each category was then used to mask the original L1 vs E1 contrast.

Coordinates in Talairach space (left/right, posterior/anterior, inferior/superior negative/positive convention) for local maxima and the associated anatomical localizations from the Talairach Daemon [11] were determined using the *t* values associated with the L1 vs E1 contrast. The *t* values listed for the other two contrasts were extracted from these coordinates.

There were no significant voxels in the four categories where both E1 and L1 changed in the same direction relative to wake. The results from the six remaining categories are presented in the Table 1.

Significant voxels from the first four categories (increases or decreases in activity uniquely associated with E1 or L1) were localized to areas including the bilateral hippocampus (see Fig. 1), the left middle frontal gyrus, the bilateral inferior parietal lobule, and the bilateral precentral gyrus. Higher-order association areas were present in all of the categories. There also seemed to be lateralized effects associated with certain categories: unique L1 increases and E1 decreases in the right hemisphere, unique L1 decreases and E1 increases in the left hemisphere.

Significant voxels from the remaining two categories (reciprocal increases or decreases in activity in E1 and L1) were as follows. Some frontal regions decreased early and increased late compared to wake whereas other frontal and parietal regions increased early and decreased late compared to wake.

To verify that the fMRI data is indeed uncovering novel information that the EEG could not uncover, a spectral analysis was also performed on the EEG data. This analysis used 30-s windows that corresponded to the 30-s epochs used to sleep score the EEG. Spectral power was computed for delta (1.0–3.9 Hz), theta (4.0–7.9 Hz), alpha (8.0–12 Hz), and beta (>12 Hz). A mixed-model ANOVA was performed using SPSS 12.0 (SPSS, Inc., Chicago, IL, USA) for each band where state (W, E1, L1) was the fixed factor and subjects was the random factor (see Fig. 2). None of the omnibus *F* tests were significant for any band and none of the Least Significant Difference pairwise post-hoc tests among the three states were significant for any band.

These data suggest that the activity in anatomically identifiable, volumetric brain regions exhibit differences during sleep states that would otherwise be grouped together based on standard EEG criteria. These results serve as a proof-of-concept that fMRI can identify differences in regional brain activity within EEG-defined stage-1 sleep. While this is clearly a post-hoc differentiation, there is now nothing to stop future investigations from identifying the same substates with fMRI alone. This assumes that the differences between

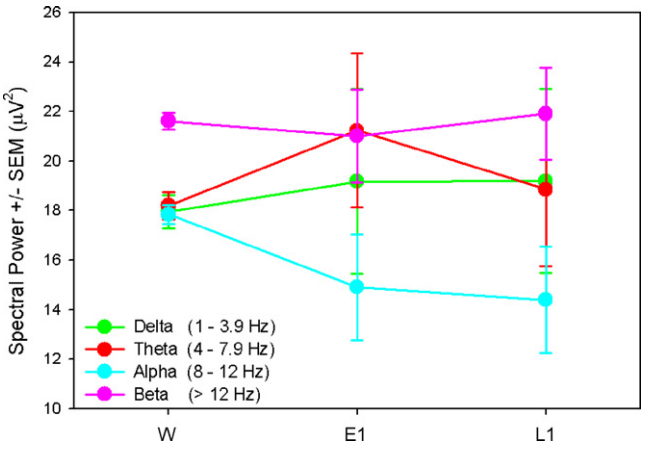

Fig. 2. EEG spectral power means for the same data used to define early and late stage 1 in the fMRI analysis.

E1 and L1 are specific to these sub-states, which may be a tenuous assumption. However, the present analysis also used wake as a baseline to combat the relative nature of the fMRI signal and this might mitigate such a limitation since subtractions between other sub-states and wake would likely yield results of at least a different magnitude.

It could be argued that such differentiation of the traditional sleep stages already exists based on EEG microarchitecture waveforms or EEG spectral analyses. The lack of any significant differences from spectral analysis in the present study seems to indicate that fMRI can potentially provide information about sleep processes that the EEG cannot. However, even if both techniques successfully differentiated early and late stage-1 sleep, an advantage of fMRI is the ability to obtain spatial information. Related to this advantage, in order to understand the functions of human sleep, it would seem important for one line of research to utilize techniques that can inherently resolve brain anatomy in three-dimensional space. This would provide the necessary information for linking the functions typically ascribed to a particular neural area during wakefulness and the nature of its activity during sleep. In addition to fMRI, other such techniques which resolve spatial information (including optical imaging and transcranial magnetic stimulation) could similarly yield novel additional information regarding heretofore unknown sleep processes.

The following additional interpretations that relate to how these differences could be relevant to the process of sleep onset and to the functions of sleep, while intriguing, are tempered by the relatively small sample size and the exploratory nature of this study. First, the majority of the areas in which activity is transiently increased in E1 can be considered part of the cortical default-mode network. This may suggest that an increase in default-mode network activity and the associated decrease in goal-directed cognition are important for sleep onset. This is also potentially consistent with increased default-mode network activity during performance lapses following sleep deprivation [5]. In other words, the same neural mechanism that underlies normal sleep onset may underline the microsleeps that presumably occur during performance lapses. Second, one of the more interesting observations is that activity in the hippocampus appears to be transiently elevated during L1. This could represent a finding with functional implications if future studies demonstrate a correlation between this activity and sleep onset mentation. Third, the lateralized change specific to each category may represent evidence of lateralized changes in human brain activity during sleep.

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