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Sound-induced perturbations of the brain network in non-REM sleep, and network oscillations in wake

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Abstract

During sleep, the brain network processes sensory stimuli without awareness. Stimulation must affect differently brain networks in sleep versus wake, but these differences have yet to be quantified. We recorded cortical activity in stage 2 (SII) sleep and wake using EEG while a tone was intermittently played. Zero-lag correlation measured input to pairs of sensors in the network; cross-correlation and phase-lag index measured pairwise corticocortical connectivity. Our analysis revealed that under baseline conditions, the cortical network, in particular the central regions of the frontoparietal cortex, interact at a characteristic latency of 50 ms, but only during wake, not sleep. Nonsalient auditory stimulation causes far greater perturbation of connectivity from baseline in sleep than wake, both in the response to common input and corticocortical connectivity. The findings have key implications for sensory processing.

Descriptors: Cognition, EEG/ERP, Sleep, Connectivity, Unconscious Processes, Normal volunteers, Auditory processing/Sensory processing

The neural basis of consciousness remains an intriguing and open question. One avenue of research into this question has been the study of the brain in sleep. The study of the sleeping brain affords several advantages—altered states of consciousness can be measured in the same individual, sleep and wake are reversible and measurable, and several modern neuroimaging methods and powerful analysis techniques can be brought to bear on the issue. Sleep and wake are global states of arousal, in that the individual and their brain are either awake or asleep, and not some state in between (Saper, Scammell, & Lu, 2005), although there may be subtle local regional differences induced by prior learning in certain parameters of brain function in sleep (Huber et al., 2006, 2007) and small but significant local and regional differences that are not necessarily linked to learning (Nir et al., 2011; Vyazovskiy et al., 2011). That sleep is a global, whole brain level phenomenon is an important point, because it implies that no understanding of the neural basis of sleep will be complete unless it comprises a comprehensive analysis of the brain network, that is, its underlying functional connectivity. Along these lines, an understanding of consciousness can be enhanced only by comparing how the brain of an individual who is awake responds to sensory stimulation, when one is typically aware of the stimulus, versus that of a sleeping individual, when one is not. These points have come to the attention of researchers of late, as evidenced by an increasing number of reports of recordings and analyses that go beyond classical sleep scoring. Broadly speaking, the literature thus far can be classified into three classes: functional magnetic resonance imaging (fMRI) studies of baseline or resting functional connectivity, fMRI studies of auditory stimulation, and electroencephalography (EEG) studies of resting functional connectivity; we note that a putative fourth class, namely, EEG studies of stimulus-induced change in functional connectivity, has yet to be reported in the literature. Nearly all of the studies we discuss below go above and beyond sleep stage scoring, in which sleep is classified into various stages—rapid eye movement (REM) sleep, nonrapid eye movement (non-REM) sleep and its substages, stages 1 and 2 (SI and SII, respectively), and the deeper slow-wave sleep (SWS). We will discuss some of the highlights of past research and along the way point the reader toward our motivations for the present study.

fMRI Studies of Baseline (Resting) Functional Connectivity in Sleep

We will begin with differences in functional connectivity between the various sleep stages and wake. There has been a surge, in recent years, of studies of functional connectivity in sleep. Picchioni et al. (2008) based their study on the premise that the blood oxygenation level dependent (BOLD) signal, with its higher spatial resolution, will reveal clearer and more spatially localized differences between wake and SI—the lightest stage of sleep—than EEG and classical sleep scoring. Indeed, the study revealed a transient elevation of hippocampal activity in late SI, and transient increase in areas of the default mode network (DMN) in early SI. The study, like others below, goes beyond the traditional EEG approach of sleep stage scoring in looking for differences between SI and wake.

Horovitz and colleagues (Horovitz et al., 2008, 2009) studied connectivity of the DMN using BOLD imaging but looked at

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PSYCHOPHYSIOLOGY

stages of sleep beyond SI. They claimed that connectivity in sleep stage SII is not appreciably different from that in wake. Larson-Prior et al. (2009) studied connectivity derived from an underlying BOLD signal (functional connectivity MRI, or fcMRI), and similarly failed to find that light sleep (SI, SII) had an effect on fcMRI of the DMN, sensory networks, or the executive control network. In sharp contrast to the reports above, Samann et al. (2011), who used a combination of fMRI and EEG as well, found a reduction in activity of certain nodes of the DMN (i.e., the posterior cingulate cortex and the retrosplenial cortex) decrease in the contribution of the medial prefrontal cortex to the DMN, and withdrawal in sleep of the brain network anticorrelated with the DMN in wake during the wake-to-sleep (SI) transition. Spoormaker et al. (2010) used graph theory to analyze fcMRI connectivity in wake and across the transition from wake to sleep, light sleep, deep SWS, and REM sleep; they found that small-worldness was largest in SWS, followed by wake, and then by light sleep (SI/SII). It is important to note that fcMRI studies, owing to the poor temporal resolution of the underlying BOLD signal, are limited to slow frequency BOLD fluctuations between 0.03–0.06 Hz, and none of these studies looked at the sleeping brain's response to sensory stimulation.

Studies of Auditory Stimulation Under Altered States of Consciousness

The loss of awareness of sensory stimulation is a key characteristic of sleep and, more generally, a loss of consciousness. This has been noted by investigators who study consciousness in brain-impaired patients who are either minimally conscious or in the vegetative state. Recent studies have focused on tying changes in connectivity with loss of consciousness in the vegetative state: one study claims a loss of top-down frontotemporal effective connectivity for auditory (mismatch) processing (Boly et al., 2011), and another claims a loss of both feedforward and feedback connectivity (King, Bekinschtein, & Dehaene, 2011). In spite of the discrepancy in the (otherwise important) details, both studies buttress the argument that the brain's response to sensory stimuli, at both the local and global levels, is likely to be a key component of the neural basis for consciousness and is, therefore, worthy of investigation.

The response to auditory stimulation has long been mined to study consciousness in the clinical setting. A number of years ago, Mantzaridis and Kenny (1997) described a novel index derived from the auditory evoked potential, termed the auditory evoked potential index or AAI, whose use is widespread in anesthesiology as a reliable indicator of potential awareness during propofol anesthesia. The AAI has since been found to be useful as a measure of awareness in sevoflurane anesthesia as well (Kurita et al., 2001). Investigators have proposed the use of auditory steady-state responses to stimuli presented at rates near 40 Hz to monitor anesthesia (Picton, John, Purcell, & Plourde, 2003). Thus, the response to auditory stimulation is now commonly used as a marker of consciousness in clinical settings. There are limitations to this approach, however: the AAI is inadequate in predicting imminent return of consciousness during decreasing propofol concentrations (Rehberg, Ryll, Hadzidiakos, Dincklage, & Baars, 2008), and the index deviates from linearity at deeper levels of anesthetic (Barr, Anderson, & Jakobsson, 2002). Given this backdrop, one could reasonably argue that sound-induced functional connectivity and network input, which have not been explored to date as measures of the depth of anesthesia, hold the potential to complement the AAI in providing a better quantitative measure of the level of anesthesia.

fMRI Studies of Auditory Stimulation in Sleep

A handful of studies have reported on the sleeping brain's response to auditory stimulation. Portas et al. (2000), using fMRI, found that similar brain areas (e.g., bilateral auditory cortex, thalamus, and caudate) were activated in wake and non-REM sleep in response to auditory stimulation, although activity in certain association areas (left parietal cortex, bilateral prefrontal cortex, cingulate gyrus) was somewhat dampened in sleep. In general, the failure to find clear and reliable localized differences in the sleeping versus waking brain's responses to sound is a strong point in favor of looking at alternatives—alternatives in technique, namely, EEG with its higher temporal resolution, emphasis on global dynamics, and built-in ability to record higher spectral modes of brain function, as well as alternatives in measure, namely, functional connectivity with an emphasis on networkwide rather than spatially localized, differences. Czisch et al. (2002) also used fMRI to study auditory processing in sleep (wake, SI, SII, and SWS), but used narrative text as opposed to simple stimuli like beeps. They observed a number of effects not observed with simpler beeps: during SI, SII, and SWS, fewer vowels activated the auditory cortex, and negative BOLD effects were observed in the visual cortex and precuneus. The authors concluded that sleep-induced changes in activity occur beyond the primary targeted sensory cortex. Their conclusion bolsters the notion that sleep-induced changes occur throughout the brain network; therefore, studying the dynamics of functional connectivity at a fast time scale across sensory stimulation is likely to yield hitherto unobserved, sleep-induced changes in connectivity.

Indeed, there have been studies of connectivity dynamics in recent years. Larson-Prior et al. (2011) used a combination of fMRI and EEG and observed a positive relationship between alpha activity and DMN together with a negative relationship to attentional networks, which is consistent with the hypothesis that increases in alpha-band power during quiet wake signal a reduction of externally directed attention. On the other hand, reduced alpha activity on visual and auditory vigilance tasks is associated with sluggish reaction times and an elevated probability of lapse (Lockley et al., 2006; Makeig & Inlow, 1993). The direction of change could depend on whether the task is conducted with eyes open or closed: decreased arousal is accompanied by increased alpha power in the eyes-open condition, whereas decreased arousal in the eyes-closed condition is accompanied by decreased alpha power (Belyavin & Wright, 1987). However, increase in alpha-band power in sleep (during which the eyes are closed) does not necessarily signal a reduction of externally directed attention: McKinney, Dang-Vu, Buxton, Solet, and Ellenbogen (2011) systematically challenged stages 2 and 3 of non-REM sleep with realistic and varied acoustic disruption, and found that sleepers exhibited markedly greater sensitivity to sounds during moments of increased alpha power. In sum, although spectral power in the alpha band has been found to be associated with vigilance and sensitivity to sensory stimulation, the direction of the relationship is not consistent across the wake-to-sleep transition. The lack of consensus regarding the role of alpha power argues, at least to us, for the need for a systematic investigation of the acoustic disruption of functional connectivity in various frequency bands in sleep versus wake, for which a technique such as EEG would be more appropriate.

EEG Studies of Baseline Functional Connectivity Dynamics in Sleep

Thus far, there have been studies comparing resting connectivity dynamics in sleep and wake. Dimitriadis et al. (2009), using a nonlinear dynamics approach, studied the dynamics of functional connectivity and the emergence of functional clusters while recording spontaneous brain activity during sleep using EEG. A graph depicting the small-world structure of the network in various stages of sleep and wake was fed into a clustering algorithm as part of a search for heretofore undescribed properties of sleep architecture. The authors made a number of exciting claims from the results of their clustering procedure, for example, anterior and posterior brain areas become isolated from one another during REM, SWS and REM are closer than SWS and SI, and so on. Ferri and colleagues also used graph theory to examine functional connectivity from EEG recordings of spontaneous activity in sleep (Ferri, Rundo, Bruni, Terzano, & Stam, 2007, 2008) and found a small-worldlike network in sleep (non-REM and REM sleep) in all frequency bands examined. Hangya et al. (2011) performed electrocorticography (EcoG) recordings in the cortex of patients with focal epilepsy, and using mutual information, another nonlinear measure of functional connectivity to study the propagation of slow wave activity (SWA), they found that SWA propagated predominantly between adjacent cortical areas. However, some long-distance connections were observed as well that could have arisen from top-down activity. Freeman and colleagues (Freeman, Holmes, West, & Vanhatalo, 2006; Freeman & Zhai, 2009) also studied EcoG data from a preoperative patient undergoing preoperative monitoring for epilepsy surgery and found steeper decrease in power spectral density in SWS, loss of beta-gamma spectral peaks, and loss of fine spatiotemporal structure in the EEG. Their work suggested that the difference in synaptic input between the two states is at the heart of these differences. It should be pointed out, however, that these studies, while seminal, used nonlinear measures of network interaction (but see Hangya et al., 2011), and only studied spontaneous activity, not stimulus-induced activity. One last point: EcoG studies have good temporal (order of milliseconds) and spatial (~1 cm) resolution, which render them ideal for the questions we are interested in, but because spatial coverage is severely limited by strict ethical guidelines, EcoG can only provide a small, spatially restricted window into the brain network and usually in very small numbers of individuals (e.g., Freeman et al., 2006, was based on data from the right inferior temporal gyrus of a single patient).

Surveying the research thus far, it is clear that studies of resting functional connectivity in sleep from the spatial (fcMRI) and dynamical (EEG) perspectives as well as studies of the brain's event-related potential (ERP) response to sensory (auditory) stimulation have yielded new insights into brain function in sleep. One expects that an analysis of stimulus-induced alterations in functional connectivity at fast time scales (corresponding to the rapid neuronal response to brief stimuli) in sleep will prove similarly fruitful. Because such an analysis has not been conducted as yet, basic questions remain unanswered. First, is there a characteristic latency at which input is transmitted to the network in the rest condition and does the value of this putative resonant resting frequency of information transmission vary depending on arousal state (e.g., in wake versus SII sleep)? Second, to what degree do simple, semantically meaningless tones perturb connectivity across the network, and, again, does this vary depending on arousal state?

In response to the questions posed above, we hypothesize the following: (a) In wake, synaptic input to the network is active whereas in non-REM sleep, synaptic input to the network is diminished (Freeman et al., 2006). The ongoing nature of network input in wake implies a characteristic latency or resonance. Interhemispheric latencies, which are over long distances in the brain, are around 50 ms; on this basis, we hypothesize that the characteristic latency in wake is less than 100 ms and it is probably not present in SII sleep. (b) For a waking brain to optimally perform its functions, that is, respond in a timely fashion to behaviorally and biologically relevant stimuli, it must be able to filter out irrelevant stimuli, such as pure tones repeated continuously every few seconds. High levels of attention and arousal associated with wake serve exactly this purpose. Thus, pure tones will not be processed extensively by the waking brain, which implies relatively unchanging levels of functional connectivity after stimulation. By contrast, in non-REM sleep and, in particular, SII sleep—a transitional stage—subjects are probably less able to filter out distracting/nonsalient sensory information. Our earlier findings, namely, that the N100 and P200 components of the auditory evoked potential (AEP) have larger amplitudes in SII sleep as compared to wake, and modulation of AEP amplitude as a function of varying sound intensity is significantly diminished in SII sleep as compared to wake (Liu & Sheth, 2009), support this point. In accord with this, we predict greater perturbations from baseline connectivity in SII sleep, and in the lower frequencies in particular (i.e., delta and theta) because of the fact that the lower frequencies are generally not involved in cognitive processes.

Methods

Subjects

Twelve healthy subjects (6 females, 24 ± 5 (*SD*) years) were recruited from the University of Houston population. The study was approved by the local human subjects committee, and informed consent forms were signed by the subjects. Polysomnography was conducted. Subjects with a total recording duration of 5,000 s or less, or subjects with less than 50 consecutive trials in either the sleep (SII) or wake state, were excluded. This meant a minimum of 15 min of continuous recording in a given state; the criterion ensures that the wake data acquired before (after) sleep are not contaminated by sleep pressure (inertia). Furthermore, it is known that SII (N2) sleep is a transitional state and 50 consecutive trials in SII sleep ensures a certain degree of stability of the SII state. Under these exclusion criteria, data from 9/12 subjects (4 females) could be used in our analysis.

Stimuli

Sounds were 1000 Hz pure tones (60-ms duration, 10-ms rise and fall times), repeated with an intertrial interval of $3{,}000 \pm 200$ ms. Software was scripted in MATLAB (The MathWorks Inc., Natick, MA), and a high-definition audio card (Realtek Inc., Hsinchu, Taiwan) was used to play the sounds. Stimuli were presented binaurally through two speakers placed 20 cm from either ear.

Task and Procedure

Subjects had to lie in a supine position on a bed in a noise-reduced room for approximately 2.5 h on average. They typically remained awake for approximately 30 min with eyes closed, after which they fell asleep on their own. After approximately 1.5 h of sleep on average, the observers woke up and stayed awake for ~30 min with

eyes closed, at which time the session was terminated. Sessions were between 13:00 and 17:00 during the day, typically later in the afternoon. Subjects passively listened to the tones throughout. No judgment regarding the sound was required.

Electrophysiological (EEG) Recording

Polysomnography (EEG + electrooculography + electromyography) recording data were acquired using a 64+ 8-channel system (ActiveTwo, BioSemi Inc., The Netherlands) throughout the recording session. EEG signals were recorded with a band-pass filter setting of 0.16–100 Hz at a sampling frequency of 512 Hz and then downsampled by a factor of four to 128 Hz (8-ms sampling period) in order to fulfill data size requirements for performing independent components analysis (ICA) in EEGLAB (Delorme & Makeig, 2004). The average referencing scheme was used; that is, the average signal of all channels was used as the reference. The average reference approach requires high electrode density and complete head coverage but otherwise provides a sound theoretical solution to the EEG reference problem and does not rely on the unrealistic assumption of electrically neutral reference sites (Hagemann, Naumann, & Thayer, 2001).

Preprocessing and Sleep Stage Scoring

Polysomnography data acquired were filtered offline in MATLAB using Parks-McClellan FIR band-pass filters (0.5–40 Hz settings) to minimize phase distortion. ICA was then performed on the filtered EEG data. For this purpose, we made use of the EEGLAB toolbox (Delorme & Makeig, 2004) in MATLAB. We used the ICA decomposition algorithm in runica.m (Infomax) provided in EEGLAB. Artifacts such as eye movements, eye blinks, muscle artifacts, and irregular spikes, which are characterized by unique spectral and spatial signatures, were manually removed. It is important to note that the manual removal was blind to the underlying state of arousal (wake/sleep). Electromyography (EMG) data were filtered between 10 and 100 Hz. Electrooculography (EOG) data were filtered between 0.5 and 100 Hz. Polysomnography data were scored automatically by a software routine called Morpheus (WideMed Inc.) and manually verified by a human scorer. In order to include most key components of the AEP, namely, the N100, P200, and the N300, a 400-ms long segment following the onset of auditory stimulation was extracted from each trial. For the purposes of symmetry, a segment of identical duration prior to the onset of stimulation (-400→0 ms) was extracted as well and constituted the baseline period. Scoring revealed a negligible number of trials in SI, SWS, and REM sleep in some subjects. Wakefulness and SII sleep were the only stages in which the predetermined minimum number (50) of trials was recorded from all nine subjects, and therefore only these two states of arousal were chosen for further analysis. The number of trials chosen for a given subject depended on the quality of sleep and on the duration of the sleep stage, and was variable across subject. Across our subject sample, 666 ± 121 trials in SII sleep and 808 ± 177 trials in wake were selected for analysis.

Data Analysis

Three measures were used: (1) Zero-lag temporal correlation (Pearson's product-moment correlation), (2) cross-correlation as a function of delay (τ), and (3) Phase-lag index (PLI).

Zero-lag temporal correlation (Pearson product-moment correlation). Cross-correlation measures the strength of linear dependence between two time series as a function of time delay between the two signals (Pereda, Quiroga, & Bhattacharya, 2005). In the case of zero-lag Pearson's product-moment correlation, the delay is zero. Corticocortical interactions between a given pair of recording sites on the scalp have a finite, nonzero delay; therefore, zero-lag correlation R_{xy} between electrodes x and y is attributable predominantly to shared (common) input to both electrodes, which arise from subcortical sources such as the thalamus and brainstem. The formula is:

$$R_{xy} = \frac{C_{xy}}{\sqrt{C_{xx}C_{yy}}}, \quad -1 \le R_{xy} \le 1,$$

where c_{xy} is the cross-covariance between signals (channel) x and y, C_{xx} and C_{yy} are the autocovariances of x and y, respectively, and R_{xy} is the correlation coefficient or coefficient of determination. We used R^2_{xy} for all our analyses, unless reported otherwise. In the present case, the length of the window of correlation was set to 400 ms. That is to say, for each stimulated trial, we estimated the instantaneous correlation values between a given pair of signals x and y from 0→400 ms following the onset of the auditory stimulus (poststimulus; note the window includes key components of the evoked potential, namely, the P50, N100, P200, and the N300), and for the purposes of equivalence, from –400→0 ms prior to stimulus onset (baseline). The resultant correlation between each pair of electrodes was averaged across all trials. For each subject, we measured the zero-lag correlation strength on each condition separately (baseline/poststimulus × SII sleep/wake). Thirty-six ($n = 9$) symmetric 64 × 64 matrices were obtained and used for statistical comparisons. Correlation coefficient values were converted using the Fisher's z transformation, following which statistical parametric tests were performed.

Cross-correlation as a function of delay (τ). Cross-correlation $R_{xy}(\tau)$ as a function of time delay τ measures the static or stationary linear interaction between a given pair of time series across a range of latencies τ. Notably, if the common source to two receiving regions contains significant autocorrelation at a nonzero latency τ, a significant cross-correlation between the two receiving regions will be observed. In the present context, if one finds clear differences in the cross-correlogram at a nonzero latency between wake and SII sleep, it implies either a difference in functional corticocortical connectivity between the two states or in the common input at that latency. The value of τ that maximizes this function can be taken as an estimate of typical intracortical delay, and to the extent that cross-correlation is a measure of functional corticocortical connectivity, it is a measure of stationary (or time-invariant) connectivity (Pereda et al., 2005). If *x(t)* and *y(t)* are zero-mean and have unit variance, the cross-correlation between them is calculated as $XCorr_{xy} = \frac{1}{N-\tau}\sum_{k=1}^{N-\tau} x(k+\tau)y(k)$, where N is the total number of samples in x and y and τ is the time delay between x and y. In our case, $XCorr_{xy}$ was normalized so the autocorrelation at zero-lag was 1. Then we took the square of $XCorr_{xy}$, which ranges from 0 to 1.

The cross-correlation function between two time series *x(t)* and *y(t)* for each pairwise connection $x - y$ (e.g., FP1 – FP2) was calculated over the 400-ms window just prior to stimulus onset. Tones occur 3,000 ± 200 ms apart, and for the 400-ms period prior to stimulus onset, the last tone occurred 2,600–3,000 (± 200) ms ago. Therefore, one can reasonably assume that the brain's

response to the pure tone will have died out by that time. We conducted an analysis of wide-sense stationarity (WSS) to confirm our intuition; specifically, if the following constraints on its mean function, $m_x(t) = m$, $\forall t \in$ [–400,0]ms (where $m_x(t)$ is the mean signal at time t of the EEG signal recorded on channel x) and the autocorrelation function, $XCorr_x(t1,t2) = XCorr_x(t1–t2)$ (where $t1,t2 \in$ [–400,0]ms), are satisfied. Our analysis found that the first and second moments did not vary statistically with respect to time over the 400-ms period immediately preceding stimulus onset. By contrast, we expected, on the basis of knowledge about the evolution of the AEP, that the 400-ms poststimulus period would not be WSS, and statistics confirmed as such. On the basis of the results of the analysis, we calculated cross-correlation as a function of delay for baseline alone.

Phase-lag index. PLI is a measure of nonlinear dependence between two nonstationary, oscillatory signals that is relatively free of volume conduction effects (Stam, Nolte, & Daffertshofer, 2007). PLI is a mathematical measure of the consistency in the difference of the moment-by-moment phases of two time series or signals. It is thus a measure of nonstationary functional connectivity and is based on the concept of phase synchronization or phase coherence, which is a measure of the phase-locking between two nonstationary, noisy signals. There are three stages of the computation:

1. Compute the instantaneous phase at each time point t of each (64 total) time series utilizing the Hilbert transform;
2. Compute the difference in phase, or phase-lag, between all $\binom{64}{2} = 2{,}016$ pairs of signals for all t;
3. Compute PLI for each pair of signals during baseline and poststimulation. The mathematical formula for PLI is as follows:

$$PLI = |\langle sign[\Delta\varphi(t_k)]\rangle|,\ k = 1, 2, 3\ldots, \text{where} -\pi < \Delta\varphi(t_k) < \pi$$

(Stam et al., 2007).

$\Delta\varphi(t_k)$ is the phase lag between two time series at time point t_k and is defined as $\Delta\varphi(t) = \varphi_1(t) - \varphi_2(t)$, where $\varphi_1(t)$ and $\varphi_2(t)$ are the instantaneous phases of the time series 1 and 2, respectively. PLI ranges between 0 and 1. PLI between each pair of channels was estimated for each subject under each condition for the following four frequency bands: delta (0.5–4 Hz), theta (4–7 Hz), alpha (8–13 Hz), and beta (13–30 Hz).

Networkwide mean, variability, and diversity of correlation strength/PLI connectivity

Mean and variability during baseline and following stimulation. For each subject, the correlation coefficient for a given pairwise correlation was computed for each trial and then averaged for a given condition (baseline/poststimulation) and arousal state (wake/SII sleep). For each subject and condition, all $\binom{64}{2} = 2{,}016$ mean correlation coefficients were then averaged. The standard deviation of all $\binom{64}{2} = 2{,}016$ correlation coefficients over all trials of a particular condition and state was computed as well.

Mean and diversity of change across stimulation. For each subject, and for each one of the $\binom{64}{2} = 2{,}016$ pairwise correlations, we subtracted the correlation strength during baseline from that following stimulation, that is, $\Delta Corr = Corr_{post} - Corr_{pre}$. We then computed the mean and standard deviation of the change $\Delta Corr$ across all $\binom{64}{2} = 2{,}016$ electrode pairs for wake and SII sleep separately. It bears mention that the diversity of change across stimulation (standard deviation of $\Delta Corr$) is not the same as change in variability, which is simply subtracting the variability in correlation strength at baseline from the variability following stimulation. Variability of change measures the diversity or range of change in correlation strength arising as a result of auditory stimulation. Identical procedures were applied to compute mean, variability, and diversity of PLI values. Statistical comparisons of network mean and variability of correlation or PLI strength between states (wake vs. SII sleep) in the baseline period, following stimulation, or across stimulation were performed using two-tailed, paired t tests.

Change in individual pairwise correlation/PLI connectivity. For each pairwise correlation, we measured the change in the strength of the correlation/PLI connection across stimulation for each subject. We evaluated the statistical significance of the change in strength of each given correlation [$\binom{64}{2} = 2{,}016$ in all] using a two-tailed t test with a false discovery rate correction for multiple comparisons. If the change across stimulation in the strength of a particular correlation was significant using the test stated above, then the direction of the change was determined (increase or decrease) and classified as such, otherwise categorized as "no change." The same procedure was performed for both wake and SII sleep. The distribution of change (increase/decrease/no change) between the two states were compared using a chi-square (χ^2) test and, when required (i.e., when the degree of freedom was one), corrected using the Yates correction for continuity.

Correlation/connectivity versus distance. A human head model was built by using the electrode-localization information provided by EEGLAB. The model was simulated as a 3-D sphere where the 64 electrodes used in our recording were spots on the surface of the sphere. The distance between any two electrodes on the scalp was the geodesic distance between them along the sphere, normalized to the radius of the sphere. The distribution of correlation strength/PLI values as a function of interelectrode distance was calculated for SII sleep and wake. Statistical comparisons of the spatial distributions of correlation/PLI connectivity values in sleep versus wake were performed using the Kolmogorov-Smirnov test.

Cross-correlation versus delay. We examined if there was a local maximum in the cross-correlation function (τ in range 47–55 ms) of each of the $\binom{64}{2} = 2{,}016$ connections. For each subject ($n = 9$) and connection, the cross-correlation function as a function of time delay (τ) was fitted with a power function ($\hat{Y} = a \times X^b$, where Y is the cross-correlation function as a function of τ and X is the interelectrode time delay τ), and the residue was examined for local maxima using the function *findpeaks* in MATLAB. If a local maximum was obtained for a value of τ between 47–55 ms for all 9/9 subjects, then the given connection was considered to contribute to the peak in the cross-correlation function.

Results

Although the terms cortical network and network connectivity are used interchangeably to describe the results of our analyses, the actual measures are on cortical sensors. In other words, a network of 64 channels or sensors on the scalp is defined here as a measure of cortical network connectivity. The results are divided into three parts. In the first part, we present results of zero-lag (or Pearson's product-moment correlation) correlation strength, a measure of common input to the cortex. In the second part, we present results from our analysis of the cross-correlation function as a function of delay τ. Finally, we present results from our analysis of PLI, a measure of functional cortical connectivity that is largely insensitive to volume conduction. In all cases, the 400-ms period immediately following stimulus onset was termed the poststimulus period, and for symmetry, the 400-ms period immediately prior to stimulus onset constituted baseline.

Wake Versus SII Sleep: Instantaneous Zero-Lag Correlation

We computed and compared the strengths of instantaneous or zero-lag pairwise cross-correlation (Pearson product-moment correlation) across the cortical surface in wake and SII sleep. First, we compared zero-lag correlation strength (henceforth, correlation strength) across the network in both states during baseline and following stimulation. Second, we compared stimulus-induced changes in correlation strength between SII sleep and wake. In both cases, we measured and compared overall mean and variability of correlation strength.

Correlation strength—mean and variability. For each subject, we measured the mean correlation strength across all $\binom{64}{2} = 2{,}016$ pairwise correlations for SII sleep and wake. There was no significant difference in mean correlation strength between the two states at baseline (mean across subjects—sleep: $R^2 = 0.27 \pm 0.01$; wake: $R^2 = 0.29 \pm 0.02$, $p > .38$, two-tailed).

Variation in correlation strength across the network is a measure of the range of input to the cortical network. For each subject, we further measured the standard deviation of correlations across all electrode pairs in each state. Across our subject sample, variability in baseline correlation strength did not differ statistically between the two states either ($p > .19$). Combined, our results show that the first and second moments of networkwide correlation strength in wake and SII sleep are statistically indistinguishable.

Change across stimulation. In contrast, auditory stimulation caused a significantly greater change in both the mean (Figure 1A) and diversity (Figure 1B) of correlation strength (i.e., post- versus prestimulus correlation values) in SII sleep as compared to wake. A between-state comparison of the average change in correlation strength resulting from the auditory stimulus found a significantly greater change (decrease) in SII sleep ($\Delta R^2 = 0.01 \pm 0.00$) than in wake ($\Delta R^2 = 0.0026 \pm 0.00$; $t(8) = 2.97$, $p < .05$). A between-state comparison of the degree of diversity of the stimulus-induced change in correlation strength also revealed a greater range of change in SII sleep (0.05 ± 0.01) across the resting baseline $\rightarrow$ poststimulus transition than that in wake (0.02 ± 0.00; $t = 7.28$, $p < .001$, paired). Thus, alterations in poststimulation correlation strength relative to prestimulus baseline were significantly greater in SII sleep. In sum, the external auditory stimulus causes a larger change in correlation structure across the cortical surface in SII sleep than in wake.

The differential effect of stimulation on overall correlation structure in wake versus SII sleep was corroborated further. We statistically compared the value of each pairwise correlation before (baseline) and after stimulation across our subject sample. The correlations that showed a significant increase or decrease in strength following stimulation were separated. As Figure 2 clearly illustrates, stimulation did not significantly alter a single one of the $\binom{64}{2} = 2{,}016$ correlations in wake; in contrast, stimulation significantly increased $452/2{,}016 = 22\%$ of all correlations and significantly decreased $701/2{,}016 = 35\%$ of correlations in SII sleep. The more frequent stimulus-induced change in correlation structure in SII sleep versus wake was significant (χ^2 goodness of fit test, $\chi^2(2) = 2{,}693.5$, $p < .0001$) as was the fact that decreases in the strength of individual pairwise correlations were more frequent than increases (χ^2 goodness of fit test, $\chi^2(2) = 53.3$, $p < .0001$). As Supplementary Figure 1 illustrates, there was a clear difference in the spatial distributions of pairwise correlations that increased versus decreased across stimulation: the increases were widespread across the scalp but were concentrated bilaterally in the occipital and parieto-occipital regions of the cortex; in contrast, the decreases were confined to the anterior parts of the cortex, namely, the bilateral frontal and temporal areas. In sum, external auditory stimulation has a dramatically different effect on the magnitude of common, shared input to a vast swath of the cortical network in SII sleep, and an appreciably smaller effect in wake.

Wake Versus SII Sleep: Cross-Correlation as a Function of Temporal Delay (τ)

Zero moment correlation is instantaneous correlation at zero delay and is likely to mainly reflect common sources of input to the pair of recording sites on the scalp. On the other hand, the correlation structure between two time series, that is, electrodes at *nonzero* latencies, is likely to mainly (but not entirely) reflect (linear) neural interaction or functional connectivity. Figure 3 plots cross-correlation $[R^2_{xy}(\tau)]$ as a function of temporal delay τ in wakefulness and in SII sleep in baseline for each subject. As the figure shows, the cross-correlation functions in SII sleep exhibit a rapid, nearly exponential decline with delay. In contrast, the cross-correlation functions in wake do not show a strictly monotonic decline with delay; in particular, at short delays, the cross-correlation functions in wake deviate away from a monotonic profile and appear to oscillate, as highlighted by the discernible peaks at ~50 ms (the range across our subject sample was from 47 ms to 55 ms) and its harmonic overtones. We further probed each individual connection, $\binom{64}{2} = 2{,}016$ in all, and selected the ones that showed a local maximum in the range 47–55 ms for all nine subjects in our sample. Figure 3, insets, and Supplementary Figures 2A and 2B, respectively, illustrate the connections that showed a local peak at ~50 (47–55) ms (932/2,016 connections or ~46% of the total) and at ~100 (95–103) ms in wake; by and large, the connections that exhibited a local peak in the cross-correlation function at 50- and 100-ms delays were connections within the (mainly central or medial) frontoparietal areas of both cortical hemispheres (these could also reflect activity in auditory cortex), and these pairwise cross-correlations were nearly all negative. In sum, during wake but not sleep, the anterior (or possibly primary

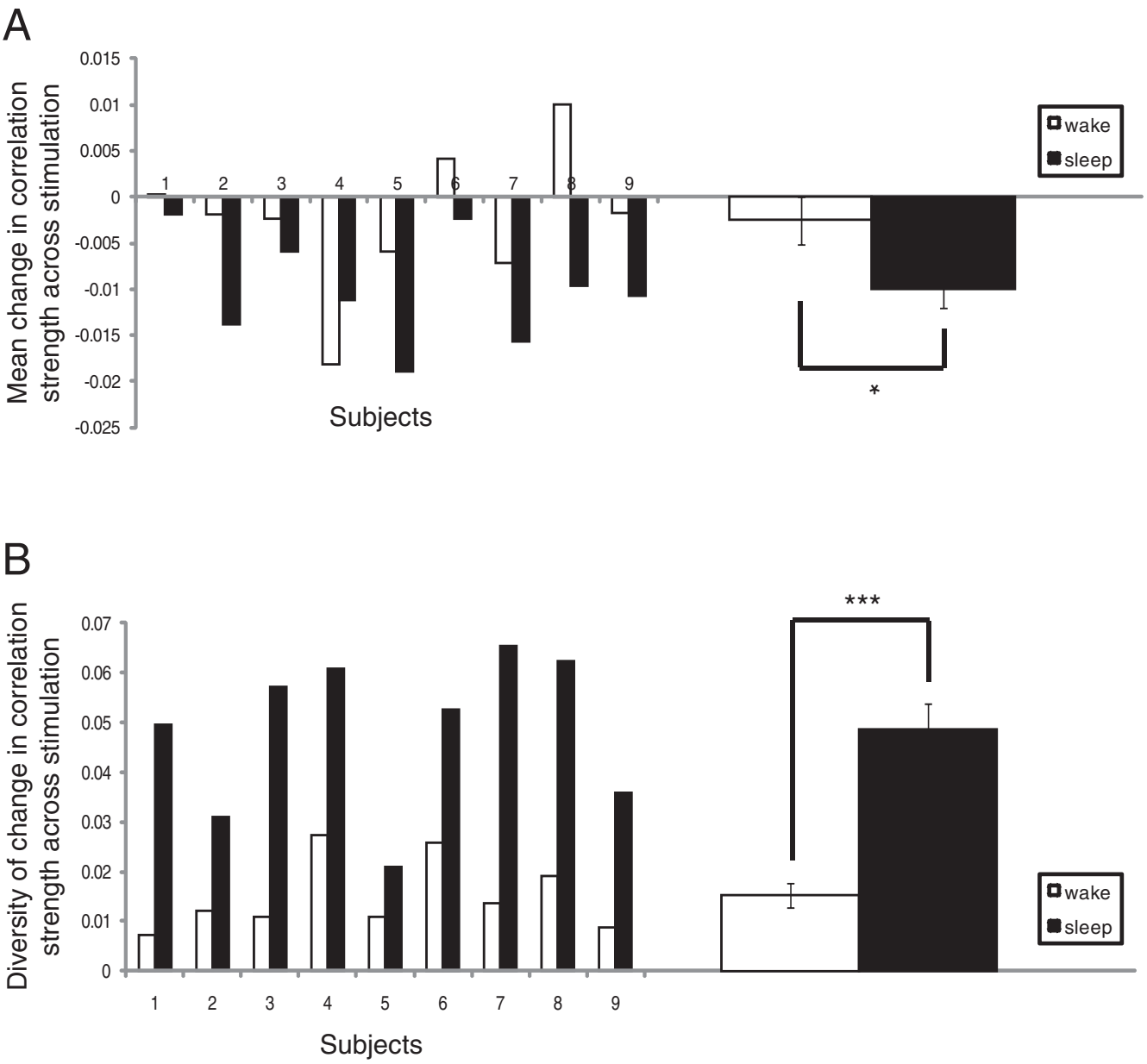

Figure 1. A: Change in mean correlation strength across stimulation, namely, difference between post- versus prestimulus baseline correlation values, in wake (white) and SII sleep (black) for each subject (thin bars on left) and the overall grand mean across all subjects (thick bars on right). B: Diversity of the change in correlation strength across stimulation in wake (white) and SII sleep (black) for each subject (thin bars on left) and the grand mean (thick bars on right). All nine subjects exhibited greater variability of change in correlation strength across stimulation during the wake state.

and secondary auditory) areas of the cortex strongly interact with one another at delays of ~50 (and 100) ms.

Wake Versus SII Sleep: PLI

We further studied functional connectivity, namely, the study of functional interactions directed at identifying statistical interdependencies between physiological time series recorded from different brain areas, using a different measure called phase-lag index. PLI is a measure of statistical interdependencies between time series, which reflects the strength of the coupling and takes into account nonlinear dependencies as well as linear ones (Stam et al., 2007). PLI between pairs of electrodes across the cortical surface was measured over four frequency bands: beta (β, 13–30 Hz), alpha (α, 8–13 Hz), theta (θ, 4–8 Hz), and delta (δ, 0.5–4 Hz), in baseline and following stimulation. Just as in the case of zero-lag correlation, we compared overall PLI connectivity values across the scalp between wake and SII sleep in baseline and following auditory stimulation.

Mean PLI strength. For each subject, we measured the baseline mean PLI strength across all $\binom{64}{2}$ connections of the cortical network in SII sleep (grand means—delta: 0.74 ± 0.00; theta: 0.73 ± 0.05; alpha: 0.70 ± 0.00; beta: 0.65 ± 0.00) and wake

(grand means—delta: 0.72 ± 0.01; theta: 0.71 ± 0.01; alpha: 0.75 ± 0.01; beta: 0.63 ± 0.00) (Figure 4). Functional connectivity strength in the theta, alpha, and beta frequencies differed significantly between sleep and wake (theta $t(8) = -3.54$, $p < .01$; alpha $t(8) = 7.40$, $p < .001$; beta $t(8) = -4.63$, $p < .01$). It is important to note that the sign of the difference was not uniform: Baseline functional connectivity in the alpha band was higher in SII sleep than in wake, but lower in SII sleep in the remaining frequency bands.

Within-subjects variability in baseline PLI strength at each frequency band was computed over all electrode pairs (Figure 5). Statistical tests revealed that only in the alpha frequency band did networkwide variability in baseline functional connectivity differ between the two states: baseline PLI strength in the alpha band was significantly less variable in SII sleep (0.07 ± 0.00) than wake (0.10 ± 0.00; $t(8) = 10.46$, $p < .001$).

Change across stimulation. Auditory stimulation caused negligible change in the overall mean and variance of (PLI) functional connectivity across the cortical network in wake and in SII sleep. Moreover, the diversity of change in PLI strength across stimulation in the two states did not differ either (Supplementary Figure 3).

We further compared pre- and poststimulus strengths of each individual connection. The results (Figure 6) show that stimulation failed to significantly alter the strength of any of the connections in wake, whereas in SII sleep, stimulation significantly changed 170/2,016 = 8% of delta connections and 381/2,016 = 19% of theta connections. The distributions of stimulus-induced change in functional connectivity in SII sleep versus wake differed significantly in both frequency bands—delta: $\chi^2(2) = 185.7$, $p < .0001$; theta: $\chi^2(2) = 469.8$, $p < .0001$. Further analysis revealed that the overwhelming majority of connections that did change across stimulation were stronger, not weaker, after stimulation in both the delta (121/170 = 71%; $\chi^2(1) = 29.7$, $p < .0001$) and theta (356/381 = 93%; $\chi^2(1) = 285.8$, $p < .0001$) frequency bands. The spatial distribution of the changing connections was informative as well (Supplementary Figure 4): The delta connections whose strength increased were concentrated primarily in the occipital and parieto-occipital areas of both hemispheres, whereas the few connections that decreased lay in the right frontal area (which could also correspond to activity in the auditory cortical areas); the theta connections whose strength increased were in bilateral frontal areas and overlapped in part with the delta connections that increased insomuch as both profiles included parieto-occipital areas of both hemispheres; too few connections decreased to establish a spatially focused profile. In sum, stimulation had a dramatically different effect on the low frequency functional connectivity of the cortical network in SII sleep, and virtually no effect on the functional connectivity in wake.

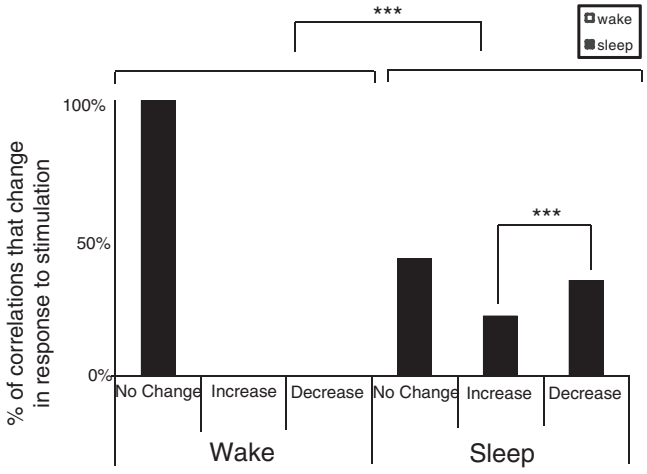

Figure 2. The percentage of $\binom{64}{2}$ correlations across the scalp that stimulation causes to significantly increase, decrease, or not change in wake (white) and SII sleep (black). None of the correlations in wake change as a result of the auditory stimulus, whereas 57% of correlations in SII sleep change. There is a significantly greater proportion of decrease rather than increase in pairwise correlations.

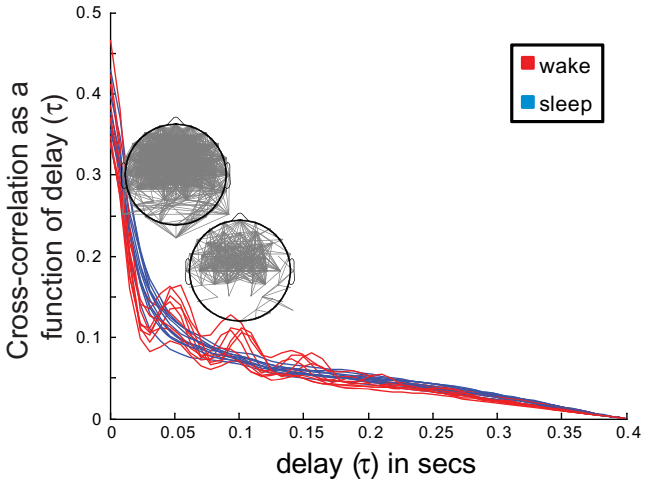

Figure 3. Cross-correlation R^2 as a function of temporal delay (τ) in baseline in wake (red lines) and SII sleep (blue lines). Each line represents data from a single subject and is the averaged across all $\binom{64}{2}$ correlations. The insets above the wake curve at 50-ms and 100-ms values of τ show the topographic distributions of correlations that have local peaks in the wake cross-correlogram at 50- and 100-ms values of τ, respectively, for all participants in our sample.

Discussion

Studies of functional connectivity in sleep in humans have focused on differences in the level of interaction between different brain regions (Bertini et al., 2007; He, Snyder, Zempel, Smyth, & Raichle, 2008; Horovitz et al., 2008; Larson-Prior et al., 2009). Here, we cast a "bird's-eye view" and investigate the dynamics of the input and connectivity to the brain network using EEG. Specifically, we explore how overall properties of the input to the cortical network and functional connectivity differ between a resting brain in (SII of non-REM) sleep versus wake, and second, we investigate how auditory stimuli—specifically, pure tones—affect each. Using zero-lag correlation (Pearson's product-moment correlation) as a measure of common input, and cross-correlation and PLI as twin measures of functional connectivity, our analysis yielded two main findings: (1) Under baseline conditions, overall cross-correlation strength in sleep across all connections decayed monotonically as a function of interelectrode delay (latency; Figure 3); in wake, on the other hand, there was a clear local maximum in the cross-correlogram at 50-ms delay (range was from 47 to 55 ms in our sample) and at multiples of 50 ms (i.e., at

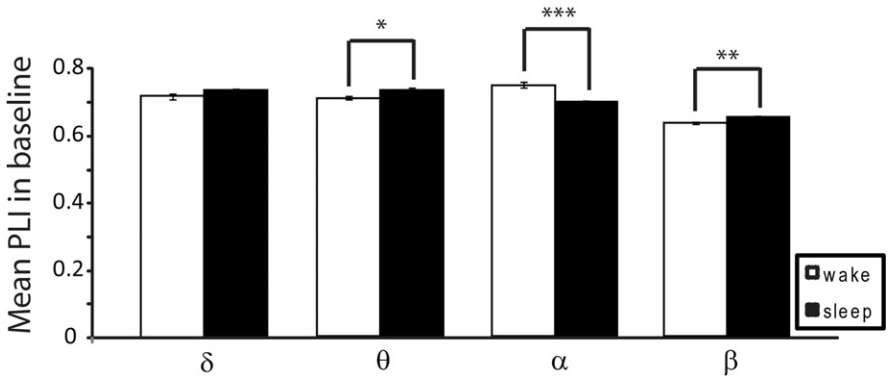

Figure 4. Mean overall baseline PLI strength in delta, theta, alpha, and beta frequencies across our subject sample in wake (white) and SII sleep (black).

100- and 150-ms delays). Thus, areas of the neural network are actively interacting with one another in the wake state at characteristic interelectrode delays of 50 ms. The networkwide delayed linear interaction appears to be a hallmark of wakefulness. (2) Auditory stimulation in SII sleep significantly altered the strength of significantly more pairwise zero-lag correlations across the network than during wake. In accord with this, stimulation in SII sleep versus wake induced significantly greater change in the overall (mean and diversity of) correlation strength across the network. Auditory stimulation had a similar differential effect on functional connectivity: Stimulation in SII sleep significantly altered the strength of a small but substantial fraction of connections (in the low frequency bands—delta and theta) whereas the same stimulation in wake failed to show a significant and consistent effect on individual connections. In sum, external sensory stimulation has a more pronounced effect on input to the cortical network as well as on functional corticocortical connectivity in SII of non-REM sleep than during wakefulness. In the remainder of the discussion, we will place the two main findings in the context of relevant literature and present knowledge about the sleeping brain.

Effect of Auditory Stimulation

Our analysis revealed that individual zero-lag pairwise correlations as well as phase-lag index measures of pairwise connectivity in the delta and theta frequency bands in SII sleep are significantly more likely to be altered by simple, pure tones than the identical interactions in wake. On the surface, these findings (Figures 2 and 6) run counter to common belief about functional connectivity in sleep and wake. However, we will argue that our findings, while surprising, do not flout conventional notions of network interaction in sleep and wake. In the end, we offer plausible, if speculative, interpretations of our finding that auditory stimulation alters functional connectivity more so in SII sleep than in wake.

As mentioned above, the findings do not appear to be in line with current dogma. Since the person is conscious of sounds while awake but not while asleep, one would naively think that this will be reflected in the functional connectivity between involved regions; that is to say, change in functional connectivity would be more common in wake than in SII sleep. In this context, two important sets of studies are worth mention.

Studies of stimulus-induced change in connectivity. Transcranial magnetic stimulation (TMS)-induced activation of the premotor area during non-REM sleep was found to cause a strong initial response at the stimulation site that was rapidly extinguished after about 100 ms and did not propagate beyond the stimulation site (Massimini et al., 2005). By contrast, the initial response in wake was weaker but lasted for an additional 300 ms, and the activity propagated within and across hemisphere in the prefrontal and posterior parietal areas. One assumes that the findings will be similar if instead the auditory cortex were stimulated with a TMS pulse, which would appear to run counter to our findings. However, there are two important caveats. First, the study compared wake with non-REM sleep, which contained stages SII and SWS. This is potentially an important difference: one could argue that the breakdown in effective connectivity occurred in SWS rather than in SII

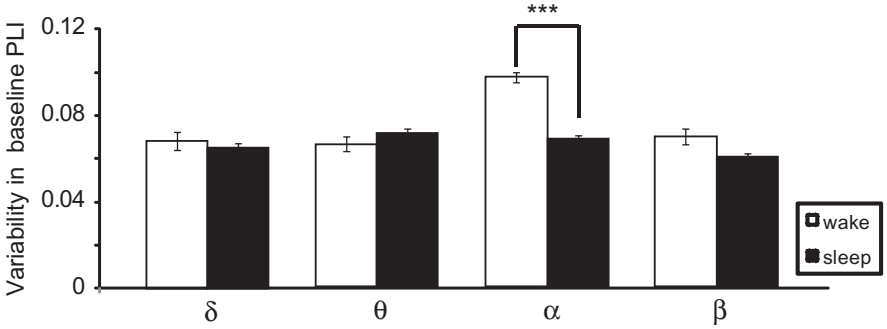

Figure 5. Within-subjects baseline variability (standard deviation) of PLI strength (delta→beta frequencies) in wake (white) and SII sleep (black).

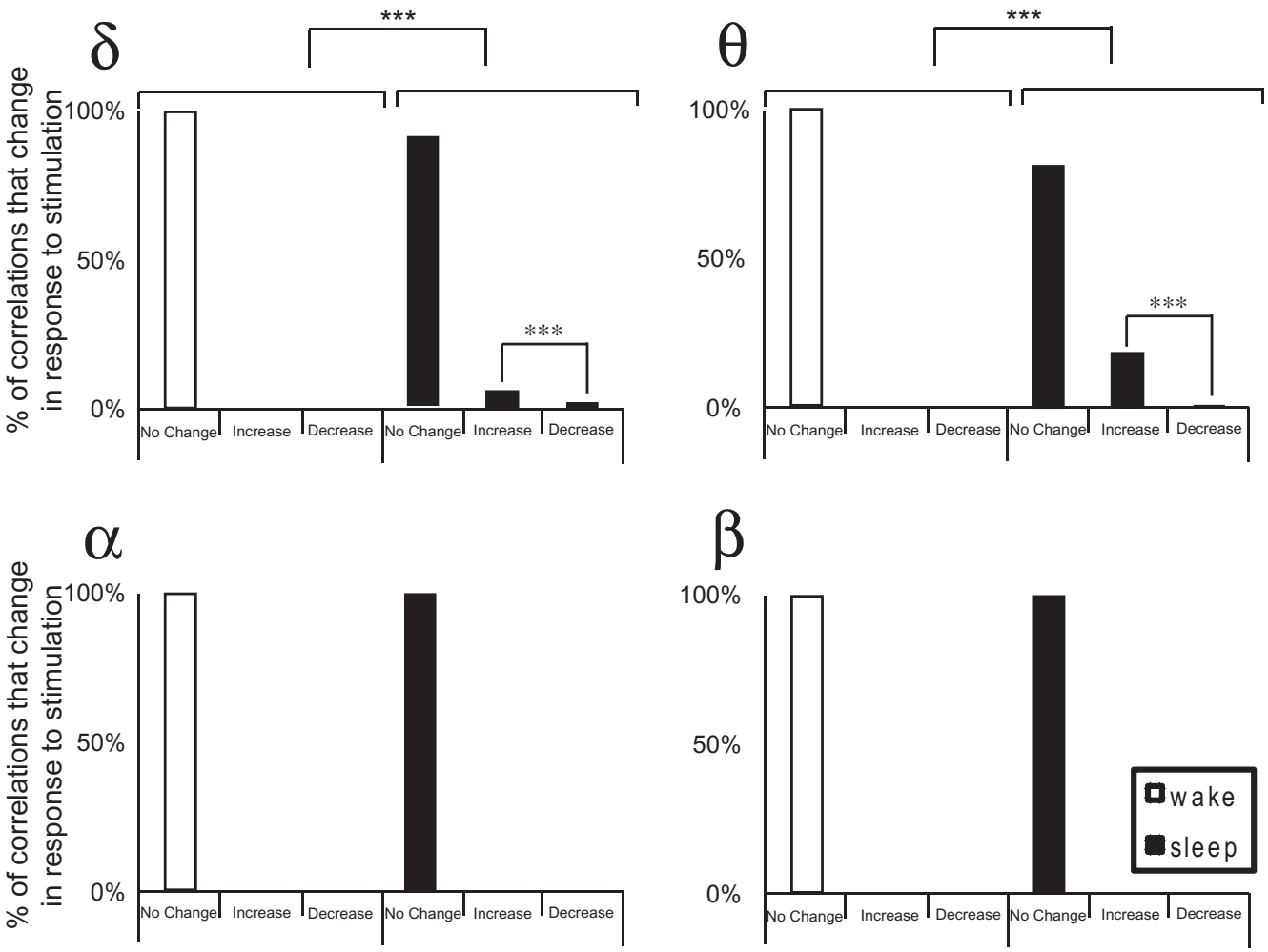

Figure 6. The percentage of PLI connections across the cortical network that stimulation did not change significantly increase or decrease in wake (white) and SII sleep (black). Note that none of the connections in wake changed as a result of stimulation.

sleep. It is possible that our results on SII sleep may fail to generalize to deeper stages of sleep. Second, the response in non-REM sleep was stronger than that in wake for about 100 ms after TMS stimulation; in addition, TMS stimulation of the cortex means that synapses from the cochlea to the auditory cortex (the N1 component occurs ~100 ms from sound onset) are short-circuited, and the delay from stimulation to cortical response is essentially zero. Thus, one could argue that, for about 200 ms or so after stimulus onset, the response is stronger in non-REM sleep, which could be reflected in a bigger change in connectivity in sleep across the 400-ms poststimulus period of the present study.

A second set of studies on the effects of stimulation on altered states of consciousness comes from clinical reports of severely brain-injured patients (Boly et al., 2004). Auditory processing was examined in patients in the persistent vegetative state (PVS), and in the minimally conscious state (MCS), and healthy controls. Brain response to simple clicks was studied using positron emission tomography (PET). Whereas the primary auditory cortex in all three sets of individuals responded to the clicks, functional connectivity between the secondary auditory cortex and temporal and prefrontal association cortices was stronger in the MCS patients than those in the PVS. Again, this study is, in many ways, complementary to ours and examines different questions. On the one hand, the spatial resolution afforded by PET studies of functional connectivity allows for the study of connections between individual brain areas and is complementary to our bird's-eye view of global connectivity at high temporal resolution. On the other hand, PET has a temporal resolution of the order of minutes and cannot be used to look for changes in functional connectivity arising from auditory stimulation occurring every 3 s (as is the case in our study). Furthermore, the studies cited were on clinical populations with severely altered consciousness whereas studies on sleep look for subtle and reversible changes in consciousness in healthy individuals. Therefore, one should not expect that stimulus-induced alterations in functional connectivity in the two cases will parallel. A propos, an overlapping group of investigators studied propofol-induced consciousness (Boveroux et al., 2010), and a later analysis of their findings concluded that anesthesia-induced unconsciousness and sleep are markedly different (Chamberlin & Eikermann, 2010).

Relationship to cognitive processing. Notably in our study, the connections whose strength the pure tones significantly alter in sleep are in the low delta and theta frequency bands. Transitioning from wake to sleep, spectral power in the low frequencies increases; as one goes deeper into successive stages of non-REM

sleep, the amount of power in the low frequencies increases even further until it peaks in SWS (Anch, Browman, Mitler, & Walsh, 1988; Dumermuth & Lehmann, 1981; Steriade & McCarley, 2005). Functional connectivity, as computed using PLI, is different from spectral power amplitude; nonetheless, it may be more than sheer coincidence that stimulation during sleep only alters low frequency connections. In this regard, it is of interest that during vigilance tasks, theta-rich EEG is associated with reduced arousal (Mackworth, 1970), deteriorated stimulus detection (Beatty, Greenberg, Deibler, & O'Hanlon, 1974; Morrell, 1966), and reduced sound sensitivity (McKinney et al., 2011). On the flip side of the coin, cognitive processing is generally associated with changes in network parameters but at frequencies beyond the theta band (Engel, Fries, Konig, Brecht, & Singer, 1999; Fries, Roelfsema, Engel, Konig, & Singer, 1997; Singer & Gray, 1995). In this context, our finding implies that, even though auditory stimulation alters functional connectivity in SII sleep more than in wake, the change is likely not associated with cognitive or conscious processing of the sound.

SWS versus SII sleep. SII sleep, at least early in sleep, is a transitional stage of sleep that occurs after wake and before the transition to deeper, slow-wave sleep. In comparison with SII sleep, the brain in SWS processes external stimuli far less. As a result, the organism in the wild is in potentially a more dangerous situation in SWS. Thus, one may consider SII sleep to be a crossroads between deep, more restful sleep on the one hand and rapid arousal, or even awakening, on the other. From this view, the brain network in SII sleep ought to be highly attuned to sounds, whereas the brain in SWS is not. A straightforward prediction from this line of thinking is that sound will alter measures of correlation and functional connectivity in SWS far less than what we observed in SII sleep.

Simple versus complex stimuli. Stimulus complexity is likely to play a role in our result as well. The waking brain clearly distinguishes between simple, otherwise meaningless stimuli like pure tones that do not demand a behavioral response and complex, cognitively demanding stimuli laden with semantic meaning that often do. By contrast, in SII sleep, behavioral response to stimuli is not even a possibility, and the capacity to distinguish complex from simple sounds is likely to be compromised. Perrin, Bastuji, and Garcia-Larrea (2002) used an electrophysiological marker of linguistic discordance, the N400 wave, to investigate how linguistic and pseudolinguistic stimuli are categorized. During wake, the N400 was greater for pseudowords than for real but semantically incongruous words, relative to congruous words. However, in SII sleep, the amplitudes were unaffected by discordance, and the sleeping brain responded to all sounds indiscriminately. The brain's inability to discriminate subtle but important nuances in sound in SII sleep suggests that the filtering mechanism (perhaps attention) that is active and functioning in wake shuts down in SII sleep. An example of this comes from the auditory sensory gating paradigm: two auditory clicks are presented in quick succession and the AEP to the second click of the pair is typically suppressed or gated in wake. The amplitude of the P50 and N100 components of the AEP in response to the paired-click stimulus are not suppressed in SII sleep, suggesting that the filtering mechanism is inactive in SII sleep (Kisley, Olincy, & Freedman, 2001). Bolstering this point from a different angle, Liu and Sheth (2009) showed that the intensity of a pure tone significantly modulates the amplitude of the P200 component of the AEP in wake, but does not in SII sleep. Phillips, Schei, Meighan, and Rector (2011) similarly found from recordings of rat cortex that, while all AEP components increased in amplitude with increased stimulus intensity during wake, this was not the case in quiet non-REM sleep. Thus, while the brain in sleep—SII sleep, in particular—does respond to auditory stimuli, its response is indiscriminate. The presumed existence of a filtering mechanism that is active in wake and dormant in SII sleep is consistent with the idea that only signals carrying high information content engage the brain network in wake but that in SII sleep, a more widespread network is activated. In this light, it is not unreasonable to expect that the processing of pure tones—unlike complex speech stimuli that require extensive processing—by the waking brain is likely limited to the auditory cortex. Our report, namely, the presentations of pure tones affects significantly more connections in the theta and delta frequencies as well as pairwise correlations in SII sleep than in wake, follows from this line of reasoning. We speculate further that if the stimulus is complex and rich in semantic meaning and emotional content, the stimulus-induced change in functional connectivity will be greater in wake than in SII sleep, opposite to the present findings with pure tones. We believe our interpretations are speculative but plausible. Above all, the above predictions, namely, reduced change in functional connectivity in SWS than wake in response to tones and greater change in functional connectivity in wake than SII sleep in response to complex speech, are testable.

Oscillatory Cross-Correlation Function

We now turn our attention to the second main finding of our study. Our study of baseline functional connectivity in the time domain yielded a significant linear interaction between widely dispersed areas of the cortex at a characteristic latency of 50 (100, and 150) ms in the wake state in every single subject in our sample, but no such interaction in SII sleep in even a single subject in our sample. We do not yet know what the true significance of this wake-specific synchronization pattern is. Here, we offer a few speculations about the underlying mechanism.

Mechanism underlying oscillation. One candidate is the mu rhythm, that is, the 50-ms peak in the cross-correlogram in wake could be due to the common input of the mu rhythm. The mu rhythm is present in wake and REM sleep, but is suppressed in non-REM sleep (Duntley, Kim, Silbergeld, & Miller, 2001), which could explain the lack of peaks in the sleep cross-correlogram. However, the mu rhythm is a lower frequency (7–10 Hz) than what we found (50-ms delays, which corresponds to 20 Hz). A second candidate stems from the remarkable similarity in timing of the 50- and 100-ms peaks of the wake cross-correlogram on the one hand and typical latencies of early P50 and N100 components of the cortical auditory evoked potential on the other. This is an exciting possibility as it has the potential to relate classical ERP recordings on the one hand and functional connectivity on the other. In SII sleep, however, the peaks at 50 ms and upper harmonics vanish, while the AEP components remain unchanged or enhanced in amplitude as compared to wake, as studies including our own have shown (Liu & Sheth, 2009). A third likely candidate is cross-hemispheric coupling. Figure 3 shows that the connections that oscillate at 50- and 100-ms latencies in the wake state are generally between channels/sensors in anterior areas, both within and across hemisphere. A simple interpretation therefore is increased frontal cross-talk in the wake state, although we add a note of caution here: Our data analysis is conducted at the sensor, or scalp level, and activity at a frontal electrode does not necessarily reflect frontal

cortex activity; the frontocentral topography shown in Figure 3, inset, could arise from late auditory cortex activity patterns. Regardless of interpretation, the oscillations in the cross-correlogram in wake but not in SII sleep suggest a feature of connectivity that distinguishes wakefulness from non-REM sleep.

Connectivity dynamics. In regards to the literature on functional connectivity across the wake-sleep transition, our finding, namely, that the cross-correlogram peaks at 50 and 100 ms are confined mainly to the anterior areas of the cortical surface in wake, differs somewhat from recent findings of a breakdown in connectivity between anterior and posterior nodes of the DMN in SII (Larson-Prior et al., 2011) and SWS (Horovitz et al., 2009). However, our technique cannot spatially distinguish nodes of the DMN from nodes in the same region but belonging to other networks, for example, the dorsal attention network (DAN) or the executive control network (ECN). It is entirely possible that our finding and those showing breakdown in connectivity between anterior and posterior regions during sleep could reflect the functioning of entirely different networks. On the other hand, our finding bears striking parallels with small-scale network connectivity analyses performed on BOLD data showing that core regions of the DMN, DAN, and ECN are anticorrelated with one another in wake and that these anticorrelations are reduced in SII sleep (Larson-Prior et al., 2011): the cross-correlogram peaks at 50 and 100 ms that we found in wake were overwhelmingly negative (anticorrelated) in sign and were found mainly in the anterior areas. In sum, our findings may well provide a dynamic supplement to earlier claims of connectivity in wake.

Functional role of oscillations in wakefulness. A related question in this regard is: What purpose do these evident peaks in the cross-correlogram at 50 and 100 ms serve in the functioning of the waking brain? Electrophysiological studies of the cellular basis of learning and memory have found that interactions between neuronal populations at 50-ms latencies are crucial for the establishment of synaptic plasticity. Studies of paired-pulse facilitation and paired-pulse depression show that two spikes occurring 20–80 ms apart lead to a short-lasting but cumulative increase or decrease (depending on factors such as prior amplitude) in the amplitude of the postsynaptic current (Zucker & Regehr, 2002). Paired-pulse facilitation is known to peak at a delay of 50 ms between the two pulses (Commins, Gigg, Anderson, & O'Mara, 1998). It is possible that the peaks at 50 and 100 ms in the cross-correlogram are indicative of conditions favoring short bursts of rapid, de novo learning in the waking brain.

Conclusions

Cross-correlations across the cortical surface in wake, but not in SII sleep, peak at a characteristic delay of 50 ms. Overall, network statistics characterizing input and functional connectivity are more stable to perturbation by simple, pure tones in wake than in SII sleep. These network properties uniquely characterizing wake could well be neural signatures of the efficient processing of biologically and behaviorally relevant sensory stimuli, rapid synaptic plasticity and de novo learning, and the dynamic integration of information distributed across the cortical surface.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Figure S0: EEG channel labels.
Figure S1: Correlations in SII sleep affected by sound.
Figure S2: Baseline correlations that peaked around 50,100 ms in wake.
Figure S3: Diversity of sound-induced change in PLI strength.
Figure S4: PLI connections affected by sound in SII sleep.