Spontaneous eyelid closures link vigilance fluctuation with fMRI dynamic connectivity states

Chenhao Wang, Ju Lynn Ong, Amiya Patanaik, Juan Zhou, and Michael W. L. Chee

Fluctuations in resting-state functional connectivity occur but their behavioral significance remains unclear, largely because correlating behavioral state with dynamic functional connectivity states (DCS) engages probes that disrupt the very behavioral state we seek to observe. Observing spontaneous eyelid closures following sleep deprivation permits noninvasive arousal monitoring. During periods of low arousal dominated by eyelid closures, sliding-window correlation analysis uncovered a DCS associated with reduced within-network functional connectivity of default mode and dorsal/ventral attention networks, as well as reduced anticorrelation between these networks. Conversely, during periods when participants’ eyelids were wide open, a second DCS was associated with less decoupling between the visual network and higher-order cognitive networks that included dorsal/ventral attention and default mode networks. In subcortical structures, eyelid closures were associated with increased connectivity between the striatum and thalamus with the ventral attention network, and greater anticorrelation with the dorsal attention network. When applied to task-based fMRI data, these two DCS predicted interindividual differences in frequency of behavioral lapsing and intraindividual temporal fluctuations in response speed. These findings with participants who underwent a night of total sleep deprivation were replicated in an independent dataset involving partially sleep-deprived participants. Fluctuations in functional connectivity thus appear to be clearly associated with changes in arousal.

Significance

Functionally connected brain networks exhibit recurring connectivity fluctuations. Although such dynamic connectivity states (DCS) can be expected to have behavioral correlates, linking fluctuating connectivity with behavioral state is hampered by the use of mental probes that themselves perturb observed behavior. Using the degree of eyelid closure as a proxy for vigilance state, we were able to continuously assay behavior and constrain the myriad of possible DCS to two relevant states denoting arousal in sleep-deprived persons. Intriguingly, these two DCS had counterparts in task-based data that predicted interindividual differences in the frequency of behavioral lapsing and intraindividual fluctuations in response speed. The replication of these findings in an independent dataset should encourage further investigations into the network dynamics of mental states.

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The existence of large-scale functional brain networks is evidenced by well-defined spatial patterns of correlated blood-oxygenation level-dependent (BOLD) signal fluctuation in fMRI data (1). Recent work has shown that functional connectivity (FC) within and between brain networks is dynamic, corresponding to the observation that even while we are performing a task, our mental focus fluctuates (2). Fluctuation of fMRI-based FC occurs over tens of seconds (3, 4) and exhibits different patterns across conscious and unconscious states (5, 6). Furthermore, just as interindividual differences in stationary FC relate to variation in human behavior and cognition (7–10), it seems likely that recurring patterns (11) of fluctuating FC have behavioral significance.

Temporal fluctuations in FC can arise from conscious mental activity (12), episodes of random synchrony (3), or simply time-varying levels of physiological noise (13, 14). The association between BOLD signal fluctuation in the default mode network (DMN) and mind-wandering episodes (15–17) has prompted investigations into the behavioral correlates of spontaneous resting-state FC fluctuations (11, 18). Although these fluctuations in FC have been shown to correlate with several physiological markers, such as electroencephalogram (EEG) power, magnetoencephalography (MEG) power, and heart rate variability (19–21), their behavioral significance remains unclear.

A key obstacle to elucidating clear FC–behavioral state relationships is the difficulty in evaluating mental state without the use of an intrusive stimulus or behavioral probe. For example, in mind-wandering experiments, the experience sampling technique used to identify such epochs involves periodically probing (and interrupting) participants for meta-awareness of mental drifting (22).

To circumvent having to use probes to evaluate mental microstates, spontaneous eyelid closures (SEC) were used as a proxy for vigilance state. In sleep-deprived persons, the degree of SEC is an excellent marker of reduced responsiveness to auditory signals (23). Pronounced SEC, referring to epochs when the eyelids are closed or almost completely closed, correspond to periods when participants are less likely to respond to standardized auditory stimuli. SEC so robustly foreshadow behavioral lapses that they are commercially used for drowsiness detection (24, 25).

We recently found that time-locking FC estimation to the onset of pronounced SEC reveals accented forms of the stationary FC shifts observed in sleep-deprived healthy young adults compared with when they are well rested (26). These FC changes involve decreased within-DMN and within-dorsal attention network connectivity of default mode (DAN), as well as reduced anticorrelation between DMN and DAN (27–30). In the present work, we sought to demonstrate that spontaneous FC fluctuations in sleep-deprived persons correspond to fluctuations in arousal that coincide with pronounced SEC. Motivating this approach are the twin observations that: (i) psychomotor vigilance in sleep-deprived persons shows pronounced moment-to-moment fluctuation (31), giving rise to sufficient state variance needed for

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reliable state classification; and (ii) prolonged SEC are more likely in the sleep-deprived state. We anticipated that spontaneous SEC in the sleep-deprived state would be associated with dynamic FC (DFC) changes in the DMN and DAN, incurring both within- and between-network shifts. We additionally expected that such dynamic connectivity patterns corresponding to “low-arousal” SEC epochs would coincide with behavioral lapses during an auditory vigilance task, and that this could be demonstrated within and across participants. We tested the reproducibility of our findings with an independent dataset involving partially sleep-deprived participants. Taken together, these predictions, if true, would support the notion that specific patterns of DFC fluctuation correspond to variations in arousal level.

Results

Fluctuations in FC at Rest Are Associated with SEC in Sleep-Deprived Participants. To elucidate the different time-varying patterns of whole-brain FC, we used a sliding-window approach to compute windowed covariance matrices from BOLD time courses extracted from the 126 predefined regions of interest (ROIs) in each participant (Fig. 1, step 1; details in Materials and Methods). Each was computed over a 40-s sliding window, shifted in 2-s increments (32). To estimate recurring DFC patterns, we performed k-means clustering on the aforesaid windowed covariance matrices pooled across all of the participants (Fig. 1, step 2). Each resulting cluster centroid was taken to be the exemplary FC pattern associated with each of several dynamic connectivity states (DCS) (Fig. S1, Left). Each frame in successive time windows was thus assigned with membership to one of these distinct DCS.

We next correlated the occurrence probability of each DCS with the SEC score (1, closed; 9, open) at corresponding time windows (Fig. 1, step 3). The occurrence probability of each DCS was based on the membership of each window as determined using k-means clustering at different degrees of SEC. We found two DCS that were either positively (Fig. 2, Left) (Spearman’s ρ = 0.905, P = 0.005) or negatively (Fig. 2, Right) (Spearman’s ρ = −0.970, P < 0.001) associated with SEC scores [P < 0.05 family-wise error rate (FWE)–corrected] (see Table S1 for the state distribution of these two DCS across subjects). No other DCS were associated with SEC. A random-effects group-level analysis showed that this SEC–DCS association was significant in most subjects (P = 0.028, t = 2.20; mean Spearman’s ρ = 0.372 ± 0.535 and P < 0.001, t = −4.63 and mean Spearman’s ρ = 0.461 ± 0.373 for positive and negative SEC–DCS correlations, respectively). This finding remained robust even with different numbers of clusters k = 3, 5, and 7 and with different sliding-window lengths (SI Results and Figs. S2 and S3). Additional analyses involving a high number of clusters (k = 11 and 13) showed largely the same results (SI Results and Fig. S4).

High- and Low-Arousal States Exhibit Within-Network and Between-Network Differences in FC. Having determined how different DCS relate to eyelid closure (SEC scores), we next characterized how FC patterns differed between high- and low-arousal DCS. To this end, we gathered a pair of windowed covariance matrices from each participant corresponding to the high- and low-arousal DCS. To minimize the effects of noise and clustering error, only windows corresponding to DCS identified as “high-” or “low-arousal in more than 50% of the clustering results, and with different k values, were used. Averaged covariance matrices for high- and low-arousal DCS were thus obtained (Fig. 3, Upper, and SI Materials and Methods). Comparison between the two groups of matrices, using two-sample t tests on Fisher’s Z-transformed Pearson correlation coefficients, revealed FC differences between these DCS (P < 1E-6 FWE-corrected) (Fig. 3, Lower).
Importantly, high spatial similarity \((r > 0.85)\) between the DCS was found regardless of the window length (Fig. S3) or the number of clusters used. We then used the same approach to summarize the clustering results across different \(k\)’s (as described above) to produce averaged windowed covariance matrices associated with high- and low-arousal DCS in the task condition (Fig. 4, Left). Similar within- and between-network FC differences were observed compared with the task-free analysis (Fig. S5).

**DCS Predict Interindividual Differences in Behavioral Performance.** We next investigated if arousal-associated DCS could predict interindividual differences in vigilance performance. To answer this question, we specified an individual’s auditory vigilance task (AVT) performance using the proportion of behavioral lapses across all trials (60 min). An individual’s lapse frequency was positively correlated with dwell time in the low-arousal DCS \((\rho = 0.465, P = 0.022)\) and negatively correlated with her dwell time in the high-arousal DCS \((\rho = -0.584, P = 0.003)\) (Fig. 4, Right). The third DCS, the one identified as neither low- nor high-arousal DCS, did not significantly correlate with an individual’s AVT task performance \((r = 0.118, P = 0.583)\). These findings were obtained using cluster number \(k = 3\), but were also largely replicated using other \(k\) values (values 5, 7, and 9) (SI Results and Fig. S6).

**Fluctuations in Dynamic Connectivity and Vigilance Are Linked.** In addition to predicting individual differences in vigilance across the entire experiment, we wondered whether fluctuations in FC patterns could inform us about intra-individual fluctuation in AVT response times. To quantify fluctuations in FC with respect to the identified high- and low-arousal DCS, we computed the spatial similarity of each windowed covariance matrix to these...
states using partial correlation. We used the rank-ordered mean reaction time of all trials within each successive sliding window to quantify temporal variation in arousal (Materials and Methods).

For each participant, faster responses corresponded to periods of greater spatial similarity to the FC pattern associated with high arousal. Conversely, when there was high spatial similarity to the FC pattern associated with low arousal, participants either responded more slowly or not at all (Fig. 5). Across participants, the correlation between the similarity of DCS expression to either high- or low-arousal state, and reaction time was \( r = -0.325 \pm 0.208 \) (\( t = -7.60, P < 0.001 \)) for high-arousal state and \( r = 0.298 \pm 0.187 \) (\( t = 7.72, P < 0.001 \)) for the low-arousal state. These findings remained significant after accounting for the number of stimuli presented in each time window and autocorrelation (SI Results). Moreover, the correlation between the similarity of DCS expression to the non-SEC-associated state and reaction time was not significant (\( r = 0.047 \pm 0.146 \), one-sample \( t \) tests \( P = 0.129 \)) (SI Materials and Methods).

Replication of Key Findings Using an Independent Dataset Involving Partial Sleep Deprivation. To test the robustness of our findings, we analyzed another independent dataset of partially sleep-deprived participants using the identical steps outlined for participants who underwent a single night of total sleep deprivation. Earlier findings were replicated at all levels of analyses, including the SEC–DCS association in the task-free data and the DCS–vigilance relationship associated with AVT task performance (both interindividual difference and intraindividual temporal fluctuations) (SI Results, Replication Study, and Fig. S7).

Discussion

We studied time-varying whole-brain FC under task-free and task conditions in healthy young adults undergoing a single night of total sleep deprivation. Using degree of SEC as a proxy for level of arousal, we identified recurring FC patterns in the task-free data that conformed to high- and low-arousal DCS, respectively. These states showed systematic differences in FC. The high-arousal state was associated with greater intranetwork connectivity involving the DMN, control, and attention networks, as well as greater anticorrelation between the DMN and attention networks. Visual network, striatal, and thalamic connectivity also differed between the two states. The same two DCS could be identified after regressing out task-related signals associated with performing an AVT. Critically, we found that high- and low-arousal DCS could independently predict interindividual differences in frequency of behavioral lapsing as well as intraindividual fluctuation in response speed. At testing to their robustness, these findings were replicated using an independent dataset involving partially sleep-deprived participants.

Linking Fluctuations in FC and Behavioral State. Brain activity during task-free fMRI experiments does not remain in a stationary resting state (33, 34). It has been established that spontaneous fluctuations in intrinsic FC are not simply noise (35) and can be correlated with physiological markers, such as EEG or MEG power at different frequency bands (19, 20), as well as with heart rate variability (21). Shifts in EEG power in the α- and θ-bands correspond to changes in arousal (36–38). Although these results are of physiological relevance, they only indirectly link FC fluctuation and behavioral state, require the use of technically demanding and expensive simultaneous EEG–fMRI methodology, and are difficult to deploy for real-time behavioral assessment. In contrast, monitoring eyelid closure is simple to implement and predicts an increased likelihood of behavioral lapses (23–25, 39). As such, SEC provides readily implementable measure to connect FC fluctuation with behavioral state.

We previously showed that prolonged SEC (distinct from blinks in awake persons) in the sleep-deprived state likely represent brief sleep intrusions (microsleeps) during which responses to auditory stimuli are slow or absent. Sensory threshold elevation during sleep (40) results from reduced transmission of sensory information to higher cortical areas. Specifically, higher cortical processing of sensory inputs, necessary for speedy responses to target stimuli, is attenuated as sleep deepens and higher cortical areas become progressively more isolated from brainstem, subcortical, or primary sensory cortical inputs (41).

Sleep deprivation (27–29) and falling asleep (42, 43) have both been associated with reduced FC within the DMN, as well as reduced anticorrelation between task-positive networks and the DMN. These alterations in FC have also been observed during periods of mind-wandering in the absence of meta-awareness (33) and during eyes-closed rest compared with eyes-opened rest (44). It has been proposed that “descent to sleep” is facilitated by both reduced thalamocortical connectivity at sleep onset (45) and a breakdown of general connectivity associated with deeper, slow-wave sleep (30). Both of these processes reduce the brain’s capacity to integrate information across functional modules (30, 43, 46, 47). Anticorrelation between the DMN and task-positive networks in particular, is thought to reflect the competitive balance between internally and externally oriented cognition and is weakened in conditions of reduced consciousness (48, 49).

Indeed, persons evidencing stronger anticorrelation between the DMN and attention networks in the well-rested state appear to be more resilient to sleep deprivation (29).

These observations notwithstanding, the relationship between FC and behavior remains enigmatic. For example, although on the average decline in the DMN and DAN FC with sleep deprivation is associated with increased lapsing, the extent to which stationary FC is altered does not correlate with the frequency of behavioral lapsing (28, 29).

The current strategy of selecting polar DFC states by constraining them with a continuously observable but proxy of behavioral state (SEC) allowed us to transcend the limitations of
using static FC of limited networks to uncover FC and behavior mappings. The utility of using SEC in the context of fMRI recordings was recently explored in two studies. The first study documented differences in resting-state fMRI global signal amplitude between eyes-open and eyes-closed states to EEG vigilance (50), and the second study documented fMRI BOLD signal fluctuations to eye-closure and invasive electrophysiological recordings in primates (51). Although relevant and buttressing the claims made here, these studies did not specifically address the trine relationship between fMRI DFC, eyelid status, and vigilance behavior documented here.

Broader Implications of DCS Identification. A recent meta-analysis of resting-state FC studies found that when using sophisticated fMRI signal analysis methods, epochs containing sleep are present in up to a third of awake studies (5). Because falling asleep modulates FC, proper characterization of awake resting-state FC requires consideration of how frequently such sleep epochs occur. The present work begs the questions: What if, apart from voluntary sleep deprivation, a participant has increased dwell time in the low-arousal state? Would post hoc editing of sleepy epochs using machine-learning techniques be beneficial or would it also remove informative connectivity patterns? Patients with attention deficit hyperactivity disorder, for example, show increased variance in response times (52) that could be mirrored in increased dwell time in our low-arousal state.

Whereas the present results show an unequivocal link between specific DCS and arous/vigilance, the high dimensionality of DFC data are such that depending on the behavioral metric used, different states may be uncovered. As such, it is important to point out that although we focused specifically on vigilance, pegging a pattern classifier to other interesting mental states should be feasible, the key challenge being to find a physiological proxy for mental state of interest that can be observed without interrupting the natural flow of thought. A particularly fertile ground to explore would be heterogeneous mind-wandering states (53). Future work could also lend fresh meaning to the metaphor “changing mental gears” when speaking of transitions in mental effort.

Conclusion

By using SECs as a proxy, we tracked temporal fluctuations in behavioral states without relying on potentially disruptive mental probes. We established a direct association between two patterns of FC fluctuations and arousal.

Materials and Methods

Participants, Data Acquisition, and Preprocessing. Data from 18 participants (9 males; aged 22 ± 2 y) were included in the analyses. All participants provided informed consent in compliance with a protocol approved by the National University of Singapore Institutional Review Board. They were screened for regular sleeping habits and their sleep patterns were monitored 1 wk before the scan (SI Materials and Methods). All participants were scanned at 6:00 AM following 1 night of restricted to 5 h of nocturnal sleep on the previous night and underwent scans at 3:00 PM, (ii) the two 6-min task-free fMRI scans were performed back-to-back at the beginning, and (iii) concurrent EEG data were collected. Both task-free and task-based imaging data were preprocessed and analyzed using the same DFC method as those in the total sleep-deprivation dataset.

To derive distinct DCS, a k-means clustering algorithm was applied to all windowed covariance matrices (18 subjects × 2 runs × 156 windows per run = 5,616 windows) using city block distance as the similarity measure. To reduce redundancy between time windows and to reduce computational load, we performed subsampling along the temporal dimension to identify windowed covariance matrices with local maxima in FC variance. This resulted in a subset of 334 windows that were clustered using k-means. The optimal number of clusters (k) was determined to be nine based on elbow criterion, computed as the ratio of within-cluster to between-cluster distances, after searching a range of k from 2 to 10. Clustering was repeated 10 times with random initialization of starting centroid locations. The resulting centroids from the subsamples were then used as the starting point for clustering of all data (5,616 windows) (Fig. 1, step 2). We repeated the same procedure for different numbers of clusters k = 3, 5, and 7.

To identify SEC-related DCS, we correlated the probabilities of DCS occurrence with SEC scores using Spearman’s rank correlation. The same tapered time window used previously for fMRI data analysis was applied to the time courses of SEC scores to derive SEC ratings per window. These windowed SEC scores were subsequently binned into 8 (1-2-8-9) to estimate the probability of DCS occurrences for each SEC bin (Fig. 1, step 3). The occurrence probability of each DCS at each SEC bin was estimated as the proportion of time each windowed connectivity matrix was assigned to that DCS cluster.

Identifying SEC-Associated DFC States at Rest. DFC analyses were performed based on a predefined set of 126 ROIs, which included 114 cortical regions derived from an independent analysis of whole-brain functional organization in a large sample of 1,000 subjects (57) and 12 subcortical structures from the Automated Anatomical Labeling template (58). The 114 cortical regions were further grouped into eight intrinsic connectivity networks: the DMN, control, limbic, visual, somatosensory, temporal-parietal, ventral attention, and DAN (57).

DFC between the 126 ROIs was estimated using a sliding-window approach (11). Specifically, tapered time windows were created by convolving a rectangle (width = 40 s) with a Gaussian window (window w = 6 s). The covariance matrices of the windowed fMRI data were estimated from a regularized precision matrix using graphical LASSO methods (59, 60). L1 norm penalties were applied on the precision matrices to promote sparsity and were the group mean of individually optimized L1 penalties based on the log-likelihood of unobserved data, as previously described (11). This was repeated successively along the fMRI time course in steps of 1 repetition time (TR; 2 s), resulting in 156 windowed covariance matrices per 6-min run. We also repeated the analyses using 30-, 70-, and 100-s window lengths to ensure the robustness of our findings.

Replication Analyses Based on Partial Sleep-Deprivation Data. The partial sleep-deprivation dataset comprised of 17 participants (age = 22.2 ± 1.8, 9 males) was acquired from an independent study (SI Results, Replication Study). The participant selection criteria and experimental set-up were similar to the main dataset, except for the following: (i) subjects were restricted to 3 h of nocturnal sleep on the previous night and underwent scans at 3:00 PM, (ii) the two 6-min task-free fMRI scans were performed back-to-back at the beginning, and (iii) concurrent EEG data were collected. Both task-free and task-based imaging data were preprocessed and analyzed using the same DFC method as those in the total sleep-deprivation dataset.

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