SLEEPJ, 2018, 1–12

doi: 10.1093/sleep/zsy108
Advance Access publication Date: 29 May 2018
Original Article

ORIGINAL ARTICLE

Connectivity of sleep- and wake-promoting regions of the human hypothalamus observed during resting wakefulness

Aaron D. Boes[1,2,3,*,†], David Fischer[4,†], Joel C. Geerling[5], Joel Bruss[2], Clifford B. Saper[6] and Michael D. Fox[6,7,8]

[1]Department of Pediatrics, Iowa Neuroimaging and Noninvasive Brain Stimulation Program, University of Iowa Hospitals and Clinics, Iowa City, IA, [2]Department of Neurology, Iowa Neuroimaging and Noninvasive Brain Stimulation Program, University of Iowa Hospitals and Clinics, Iowa City, IA, [3]Department of Psychiatry, Iowa Neuroimaging and Noninvasive Brain Stimulation Program, University of Iowa Hospitals and Clinics, Iowa City, IA, [4]Department of Neurology, Berenson-Allen Center for Noninvasive Brain Stimulation, Division of Cognitive Neurology, Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, MA, [5]Department of Neurology, University of Iowa Hospitals and Clinics, Iowa City, IA, [6]Department of Neurology, Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, MA, [7]Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown, MA and [8]Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA

*Corresponding author. Aaron D. Boes, Departments of Pediatrics, Neurology, and Psychiatry, Iowa Neuroimaging and Noninvasive Brain Stimulation Program, University of Iowa Hospitals and Clinics, W276 GH, 200 Hawkins Drive, Iowa City, IA 52242. Email: Aaron-Boes@uiowa.edu.

†These authors contributed equally to this work.

Abstract

The hypothalamus is a central hub for regulating sleep–wake patterns, the circuitry of which has been investigated extensively in experimental animals. This work has identified a wake-promoting region in the posterior hypothalamus, with connections to other wake-promoting regions, and a sleep-promoting region in the anterior hypothalamus, with inhibitory projections to the posterior hypothalamus. It is unclear whether a similar organization exists in humans. Here, we use anatomical landmarks to identify homologous sleep- and wake-promoting regions of the human hypothalamus and investigate their functional relationships using resting-state functional connectivity magnetic resonance imaging in healthy awake participants. First, we identify a negative correlation (anticorrelation) between the anterior and posterior hypothalamus, two regions with opposing roles in sleep–wake regulation. Next, we show that hypothalamic connectivity predicts a pattern of regional sleep–wake changes previously observed in humans. Specifically, regions that are more positively correlated with the posterior hypothalamus and more negatively correlated with the anterior hypothalamus correspond to regions with the greatest change in cerebral blood flow between sleep–wake states. Taken together, these findings provide preliminary evidence relating a hypothalamic circuit investigated in animals to sleep–wake neuroimaging results in humans, with implications for our understanding of human sleep–wake regulation and the functional significance of anticorrelations.

Submitted: 9 November, 2017; **Revised:** 17 April, 2018

Statement of Significance

The hypothalamus has a central role in sleep–wake regulation, but the functional relationship between sleep- and wake-promoting subregions within the hypothalamus and with other brain areas remains largely unknown in humans. Using resting-state functional connectivity magnetic resonance imaging, we show that spontaneous activity in the wake-promoting posterior hypothalamus and sleep-promoting anterior hypothalamus is negatively correlated, which is interesting in light of their opposing roles in sleep–wake regulation. Moreover, functional connectivity between these hypothalamic regions and the rest of the brain relates to patterns of regional sleep–wake differences previously observed in humans with functional imaging. These findings, while preliminary, may lend further insight into the neural basis of sleep–wake regulation in humans and the functional significance of anticorrelated networks.

Key words: arousal; functional connectivity; ventrolateral preoptic; tuberomammillary nucleus; anticorrelation

Introduction

Early human observational studies on the anatomical basis of sleep–wake regulation suggested that the posterior hypothalamus promotes wakefulness, whereas the anterior hypothalamus promotes sleep [1]. These hypotheses were confirmed experimentally in rats, where lesions of the posterior hypothalamus produced hypersomnolence and lesions of the anterior hypothalamus caused insomnia [2]. Since these landmark studies, sleep–wake circuitry has been refined and extended to other regions primarily through experimental work in laboratory animals. This work has highlighted a region superolateral to the mammillary body as a key wake-promoting region of the posterior hypothalamus [2–10]. This hypothalamic region has direct anatomical connections with other wake-promoting structures, including brainstem nuclei of the ascending activating system, the basal forebrain, and the cerebral cortex [11–13]. In contrast, sleep is promoted through inhibition of the posterior hypothalamus and other wake-promoting structures. The best characterized source of inhibitory influence on this wake-promoting network is the anterior hypothalamus, specifically the intermediate nucleus\ventrolateral preoptic nucleus (IN\VLPO) and adjacent median preoptic nucleus [2, 14–19].

These sleep- and wake-promoting regions of the hypothalamus have inversely related state-dependent firing patterns such that IN\VLPO neurons are active during sleep and relatively quiescent during wake, whereas the opposite is true of the wake-promoting posterior hypothalamic neurons [3, 20–22]. The inverse firing pattern between these sites may be facilitated by direct inhibitory projections from IN\VLPO to the wake-promoting posterior hypothalamus, concentrated in the tuberomammillary region [15, 19, 23–28]. The wake-promoting posterior hypothalamus may also have an inhibitory influence on the IN\VLPO region [29–31] such that the sleep- and wake-promoting regions of the hypothalamus may be mutually inhibitory [26, 32].

It is unknown whether the hypothalamic circuitry–regulating sleep and wake in experimental animals extends to humans. Several human neuroimaging studies have contrasted sleep and wake states [33–37], identifying multiple brain regions with higher activity during wakefulness relative to sleep. However, it remains unclear if or how these large-scale patterns of sleep–wake changes seen in humans relate to sleep- and wake-promoting subregions of the hypothalamus.

In this article, we investigate the network organization of sleep- and wake-promoting regions of the hypothalamus. To do this, we use resting-state functional connectivity magnetic resonance imaging (rs-fcMRI), a technique that measures coherent fluctuations of blood oxygen level–dependent (BOLD) signal, from healthy adults while awake but resting quietly. We hypothesize that (1) the anterior and posterior hypothalamus will have negatively correlated activity, based on their inhibitory connections and reciprocal, opposing functional roles in sleep–wake regulation, and (2) connectivity with the anterior and posterior hypothalamus will predict cerebral blood flow (CBF) changes in other brain regions across sleep–wake states that have been identified in prior human neuroimaging studies.

Methods

Hypothalamic regions of interest

Regions of interest (ROIs) in the hypothalamus were selected by two anatomists with expertise in the hypothalamus (C.B.S. and J.C.G.) using a high resolution T1-weighted MRI template brain in standard MNI space (MNI152 0.5 mm voxel, available at http://fsl.fmrib.ox.ac.uk/fsl) [38]. ROIs were selected prior to conducting rs-fcMRI analyses and were aided by human histologic preparations and an MRI atlas of the human hypothalamus [39–41]. We first outlined a region in the anterior hypothalamus corresponding to the IN\VLPO. Anatomical identification of the wake-promoting region in the posterior hypothalamus was more difficult, as the precise localization of this region in the human brain remains unknown. Here, we selected a region superior and lateral to the mammillary bodies, including the tuberomammillary region, based on several lines of evidence [42] including a high concentration of inhibitory projections from the sleep-promoting IN\VLPO region [19]. ROIs were defined on the 0.5 mm brain atlas (Figure 1) and subsequently down-sampled to 2 mm voxels for rs-fcMRI analyses. Center of gravity coordinates in MNI space for the anterior hypothalamic region (IN\VLPO) are X ±4.4; Y 1.33; Z −14.67 and the posterior hypothalamic ROI coordinates are X ±6.6; Y −7.33; Z −12.67. The size of the ROIs in 2 mm space was 6 voxels (48 mm^3), 3 voxels per hemisphere, and was the same for both anterior and posterior hypothalamus ROIs.

Resting-state functional connectivity MRI analysis

Rs-fcMRI data were acquired from a large cohort of healthy participants (98 right-handed participants, 48 male, ages 22 ± 3.2 years) [43]. The time course of the BOLD signal within each hypothalamic ROI was compared with the BOLD signal of other brain voxels to identify regions with significant correlations. Rs-fcMRI data were processed in accordance with the strategy of Fox et al. [44] as implemented in Van Dijk et al. [45] and described in detail elsewhere [46, 47]. Briefly, participants completed two 6.2 min rs-fcMRI scans during which they were asked to rest in the scanner (3T, Siemens) with their eyes open (TR = 3000 ms, TE = 30 ms, FA = 85°, 3 mm voxel size (27 mm^3), FOV = 216, 47 axial slices with interleaved acquisition and no gap). Functional data were acquired at 3 mm voxel size (27 mm^3) and spatially smoothed using both a Gaussian kernel of 6

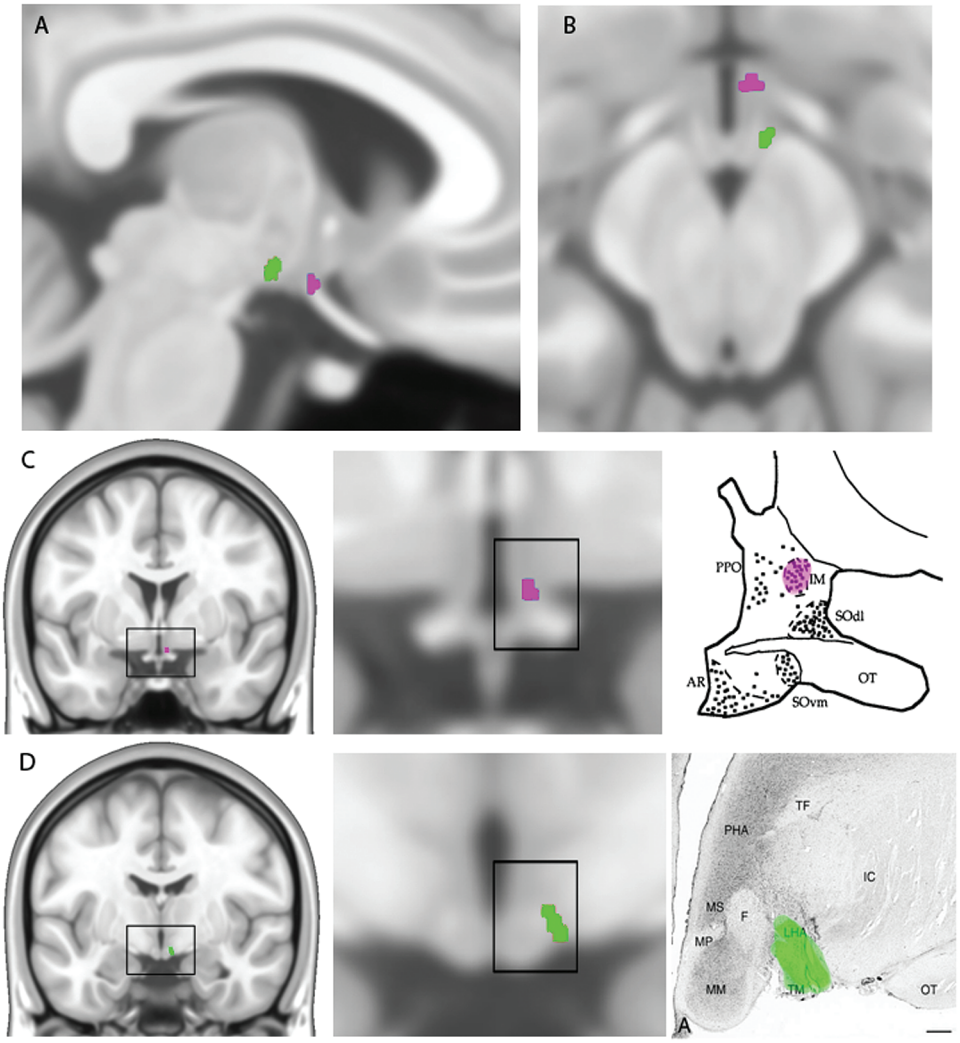

Figure 1. Hypothalamic regions of interest. Regions are shown on sagittal (A, X = 5.5), axial (B, Z = −13), and coronal (C, Y = 1; D, Y = −7) slices of an MRI. The green region is in a wake-promoting area of the posterior hypothalamus, superolateral to the mammillary bodies. The violet region is in a sleep-promoting area of the anterior hypothalamus, approximating the intermediate nucleus/ventrolateral preoptic region. (C) and (D) show progressively zoomed in views of these regions with histological sections on the right. Galanin-stained neurons are shown in (C) (modified with permission[41]) and Nissl-stained neurons in (D) (modified with permission [40]). Actual regions used for analyses were bilateral, though displayed unilaterally to facilitate comparison with underlying anatomy.

and 4 mm full-width at half-maximum. Although spatial smoothing may seem counterintuitive when investigating small hypothalamic nuclei, smoothing can help improve functional connectivity results across participants by accounting for small registration errors and anatomic variability [48–50]. The data were temporally filtered (0.009 Hz < f < 0.08 Hz) and several nuisance variables were removed by regression, including the following: (1) six movement parameters computed by rigid body translation and rotation during preprocessing, (2) mean whole brain signal, (3) mean brain signal within the lateral ventricles, and (4) the mean signal within a deep white matter ROI. Inclusion of the first temporal derivatives of these regressors within the linear model accounted for the time-shifted versions of spurious variance. Correlation coefficients were converted to normally distributed Z-scores using the Fisher transformation. A random effects one-sample t-test was used to create a network map for each hypothalamic ROI across all 98 participants. Results were considered significant at a T-value of 4.25 (p < 0.00005) as used previously for this dataset [46, 51–53] which corresponds to a false discovery rate correction of <1 percent.

Global signal regression uses a general linear model to regress out the average signal across all voxels, which includes physiological noise (e.g. cardiac and respiratory), movement-related artifact, and nonspecific signals. It was included in the primary analysis (model 1, described above) as it has been shown to improve neuroanatomical specificity, correspondence to anatomical connectivity, and remains the most common processing approach in the rs-fcMRI literature [54, 55]. However, there is concern that global signal regression confounds the ability to interpret anticorrelations [54–56]. To ensure that our results were not dependent on global signal regression, we reprocessed the data using a second model from an independent pipeline that avoids global signal regression, anatomical CompCor [57] implemented with the Conn toolbox (http://www.nitrc.org/projects/conn) [58]. Physiological and other sources of noise were estimated from the MRI data and regressed out together with artifact and movement-related covariates. The residual BOLD time series was band-pass filtered (0.009–0.08 Hz), smoothed (4 mm kernel), and linearly detrended, per default settings in the Conn toolbox. The residual BOLD time course from each ROI was correlated to that of all other brain voxels. Pearson correlation coefficients were Fisher-transformed to Z-scores to increase normality prior to a general linear model that combined data from the 98

participants, creating voxel-wise T-scores. In addition, we evaluated the spatial distribution of our findings in a third model that involved regressing out the signal from the entire hypothalamus, similar to prior efforts of local nuisance variable regression [59, 60]. This approach increases the likelihood of identifying negative correlations within the hypothalamus but it does not dictate their spatial distribution [54]. Next, in a fourth model, we explored whether nonspecific signals in the anterior hypothalamus ROI might contribute to its anticorrelation with the posterior hypothalamic region. Specifically, the anterior hypothalamus ROI lies close to the subarachnoid space (chiasmatic/suprasellar cistern) and the optic nerve, raising concern that signal from this ROI could be contaminated by movement artifact, cerebrospinal fluid (CSF) pulsation, or signal averaging with optic nerve voxels. Partial correlation analyses were conducted using the BOLD time course of these surrounding ROIs (optic nerve, subrachnoid space) to evaluate functional connectivity while accounting for these potential confounds [61, 62].

Experimental design and statistical analysis

First, the correlation between the hypothalamic ROIs was evaluated. In addition, the networks derived from these hypothalamic regions were evaluated relative to regions outside the hypothalamus implicated in sleep–wake. Global decreases in CBF occur during sleep, but some brain regions change more than others between sleep–wake states. The thalamus, brainstem, putamen, anterior cingulate, and insula in particular show much higher CBF while awake compared with being asleep [33, 36, 63–65]. The largest study to date reported 23 brain regions with higher CBF during wakefulness relative to slow wave sleep, or high sleep–wake contrast. Nine brain regions were reported with minimal differences between sleep–wake states (low sleep–wake contrast) [33]. Each PET-derived peak coordinate from this previously published analysis was converted from Talaraich into MNI space using the SPM algorithm available in GingerALE (http://www.brainmap.org/ale/). A spherical ROI was created for each coordinate using FSL (8 mm diameter, 432 mm^3), and the average BOLD time course was extracted from these spherical ROIs and correlated with the BOLD time course of each hypothalamic ROI, transformed to a normal distribution using a Fisher r-to-z, and averaged across our cohort of 98 participants. Higher correlation coefficients between hypothalamic ROIs and these PET-derived coordinates represent stronger functional connectivity, in either the positive or negative direction. CBF information across sleep–wake states came from a previously published article [33]. CBF was not measured in the same 98 healthy participants that contributed to the rs-fcMRI data.

We hypothesized that brain regions with high contrast between wake and slow-wave sleep would be (1) positively correlated with the "wake-promoting" posterior hypothalamus and (2) negatively correlated with the "sleep-promoting" anterior hypothalamus. We tested our hypothesis in three ways: (1) For our main analysis, we tested for significant connectivity between these hypothalamic ROIs and regions with high sleep–wake contrast (23 ROIs) using independent-samples T-test. We directly compared connectivity with the anterior versus posterior hypothalamus using a T-test. As a control, we then repeated these analyses using regions with low sleep wake contrast (9 ROIs) and compared the results to regions with high sleep–wake contrast (23 ROIs) using a T-test. Because this test was conducted as a group-level comparison between regions with high and low contrast, there was no correction for multiple ROIs within each group. (2) Next, we computed the proportion of regions in the high (N = 23) and low (N = 9) sleep–wake contrast groups that met the criteria of being positively correlated with the posterior hypothalamus and negatively correlated with the anterior hypothalamus. These proportions were statistically compared using a chi-squared test. (3) Finally, we tested whether the magnitude of the CBF change across the regions with high sleep–wake contrast (23 ROIs) correlated with the strength of connectivity to the hypothalamic ROIs. Specifically, we computed the Pearson correlation between the magnitude of the CBF change (Z-scores as reported in Braun et al. [33]) and the strength of correlation with the a priori ROIs in the anterior and posterior hypothalamus (Fischer Z-transformed correlation coefficients).

Posthoc descriptive analyses were included to evaluate the spatial distribution of the voxel-wise hypothalamic networks. We also performed a conjunction analysis to identify brain regions positively correlated with one hypothalamic ROI and anticorrelated with the other, as performed previously in characterizing anticorrelated networks [44]. Specifically, we identified areas both positively correlated with the posterior hypothalamus and negatively correlated with the anterior hypothalamus, as well as the converse pattern.

Results

Connectivity between hypothalamic regions

We first tested the hypothesis that the time courses of the BOLD signal extracted from the two hypothalamic ROIs would be negatively correlated (anticorrelated). Despite the close anatomical proximity of these ROIs (Euclidean distance of 9.1 mm between the center of gravity coordinates), their time courses were negatively correlated (Figure 2; r = –0.0875, T = –4.37, p = 0.00003). To explore the spatial specificity of this functional relationship, we generated resting-state functional connectivity maps for each ROI and identified peak anticorrelations within the hypothalamus. The peak anticorrelation with the posterior hypothalamus fell within our a priori ROI in the anterior hypothalamus (X 4; Y 2; Z –16; T = –6.51). The peak anticorrelation with the anterior hypothalamus fell immediately adjacent to our a priori ROI in the posterior hypothalamus (X 8; Y –8; Z –12; T = –8.05) with the adjacent voxel falling within our ROI (X 6; Y –8; Z -12; T = –7.03).

Results were similar using multiple alternative preprocessing strategies including (1) limited spatial smoothing and no global signal regression, (2) regression of signal from the entire hypothalamus, (3) regression of signal from the ventral margin of the hypothalamus along the CSF-parenchyma border, and (4) regression of signal from the optic nerve adjacent to anterior hypothalamus ROI (Supplementary Figure S1).

Hypothalamic connectivity to regions modulated by sleep–wake

Next, we tested the hypothesis that functional connectivity with these hypothalamic ROIs would predict CBF changes across 32 regions in the brain that fluctuate according to sleep–wake states, as reported by Braun et al. On average, regions with higher CBF during wakefulness were functionally connected to the wake-promoting posterior hypothalamus (mean Fisher Z = 0.091, p = 0.01) but not the sleep-promoting anterior hypothalamus (mean Fisher Z = –0.02, p = 0.39), with a significant difference in connectivity between the two hypothalamic ROIs

(T = 3.26, p = 0.01). These findings were specific to regions with high sleep–wake contrast, as regions with low sleep–wake contrast showed no connectivity to the posterior hypothalamus (T = 1.40, p = 0.2), no difference in connectivity between hypothalamic ROIs (T = 0.72, p = 0.48) and significantly less connectivity to the posterior hypothalamus than observed for regions with high sleep–wake contrast (T = 2.8, p = 0.01).

In addition to averaging across sleep–wake regions, we also examined hypothalamic connectivity to each of these 32 PET-derived regions individually (Figure 3, Supplementary Figure S2, and Supplementary Table S1). Of the 23 regions with high sleep–wake contrast, 15 showed a significant positive correlation to the posterior hypothalamus. Ten of these 15 regions were also significantly negatively correlated with the anterior hypothalamus, including regions with particularly large sleep–wake CBF changes in the thalamus, basal ganglia, insula, and anterior cingulate. This pattern of hypothalamic connectivity was specific to regions with high sleep–wake contrast, as it was not present in any of the nine regions with low sleep–wake contrast, a significant group difference (p = 0.05).

Finally, we tested whether hypothalamic connectivity could predict variance in sleep–wake CBF change within the 23 regions with high sleep–wake contrast. Regions with the largest sleep–wake CBF change were more positively correlated with the

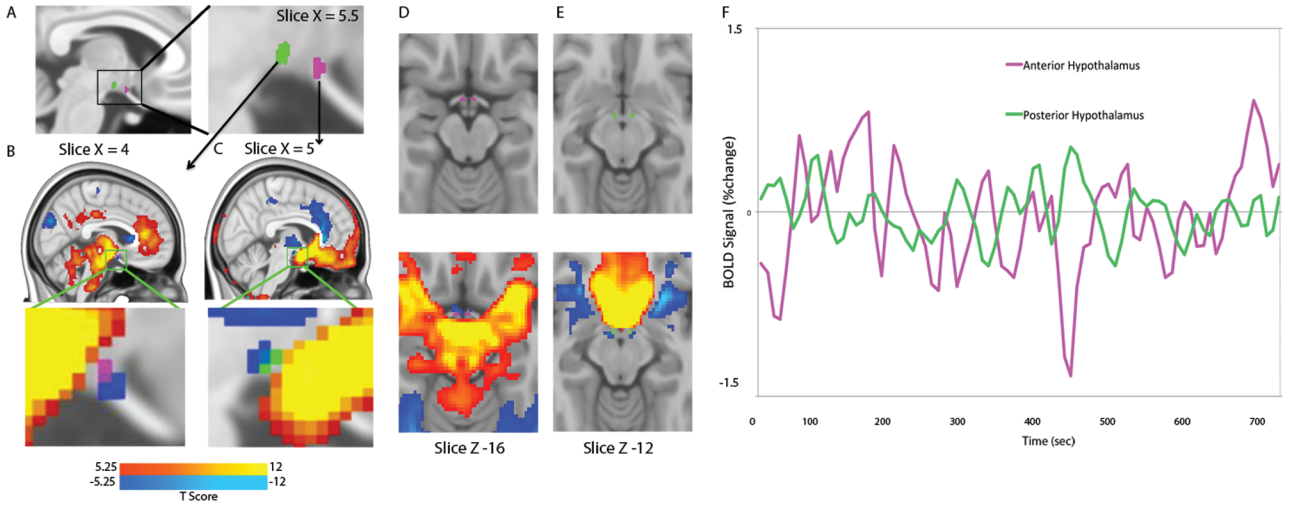

Figure 2. The anterior and posterior hypothalamus are anticorrelated. (A) Regions of interest in the posterior (green) and anterior (violet) hypothalamus show negatively correlated fluctuations in spontaneous activity, shown in (F) as a percent change in BOLD signal for a representative single participant, sampled in 3 s increments. Resting-state functional connectivity with the posterior hypothalamus showed negative correlation in the anterior hypothalamus, shown in blue ((B) and (D), axial). Resting-state functional connectivity with the anterior hypothalamus showed negative correlation in the posterior hypothalamus, also shown in blue ((C) and (E), axial). Displayed results show positive (warm colors) and negative (cool colors) correlations represented as T-scores across 98 participants that are significant at $p < 0.00005$, uncorrected.

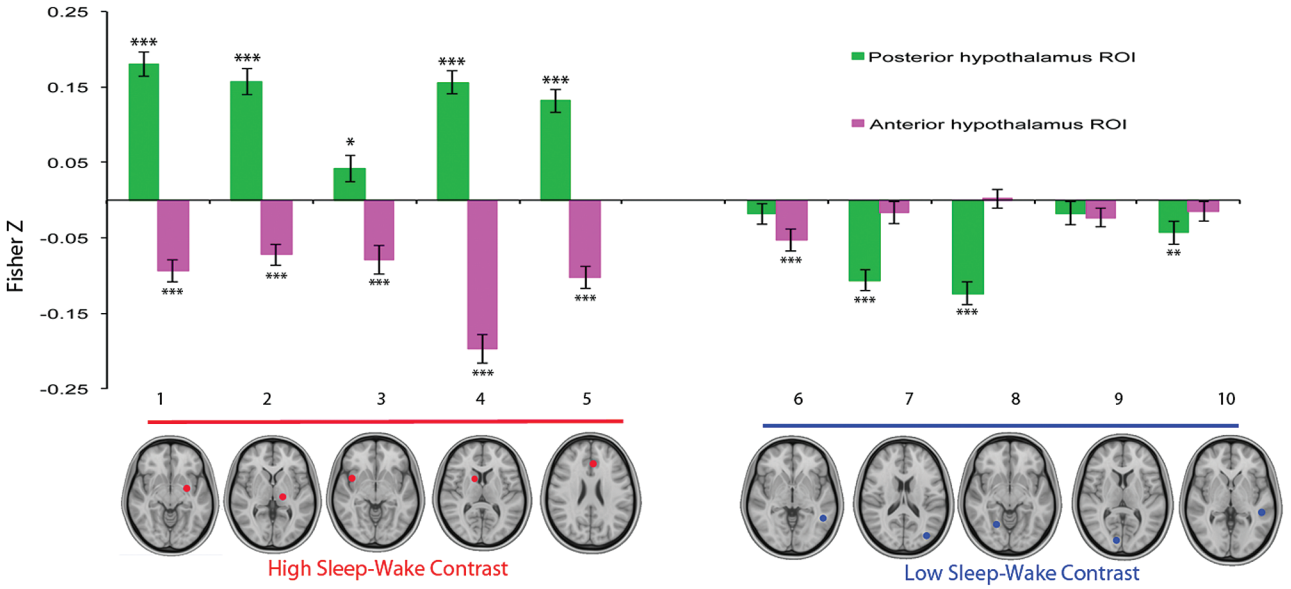

Figure 3. Regions with high versus low contrast in CBF across sleep–wake states differ in hypothalamic connectivity. Regions with high contrast in CBF in wake versus sleep (1–5, red regions) are positively correlated with the posterior hypothalamus and negatively correlated with the anterior hypothalamus. This relationship is not seen in regions with little difference in CBF across sleep–wake states (6–10, blue regions). The examples shown here were selected from a larger set of regions reported in Braun et al. [33], and displayed in full in Supplementary Figure S2 and Supplementary Table S1. Mean Fisher-Z +/– standard error of the mean across 98 participants is displayed. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

posterior hypothalamus ROI (r = 0.44, p = 0.04) and more negatively correlated with the anterior hypothalamus ROI (r = –0.41, p = 0.05). Incorporating connectivity data from both the anterior and posterior hypothalamus to create a "difference" value provided the best prediction of CBF change across sleep–wake states (r = 0.52, p = 0.01) (Figure 4).

Descriptive and posthoc analyses

Connectivity with the rest of the brain

In addition to testing our a priori hypotheses, we evaluated the spatial distribution of resting-state functional connectivity between each hypothalamic ROI and the rest of the brain in a descriptive manner (Figure 5). Several brain regions showed an inverse relationship with the anterior and posterior hypothalamic ROIs. For example, the ventral anterior insula (frontoinsular) and pregenual anterior cingulate cortices were correlated with the posterior hypothalamus and anticorrelated with the anterior hypothalamus. Other brain regions were correlated with one region but not the other. For example, the ventromedial prefrontal cortex was positively correlated with the anterior hypothalamus without a significant negative correlation with the posterior hypothalamus.

The pattern of connectivity in the basal forebrain was unique in that both the anterior and posterior hypothalamus had significant connectivity to this structure, though with complementary patterns. The posterior hypothalamus was positively correlated with the lateral basal forebrain, whereas the anterior hypothalamus was correlated with the ventromedial basal forebrain and anticorrelated with the lateral basal forebrain (Supplementary Figure S3).

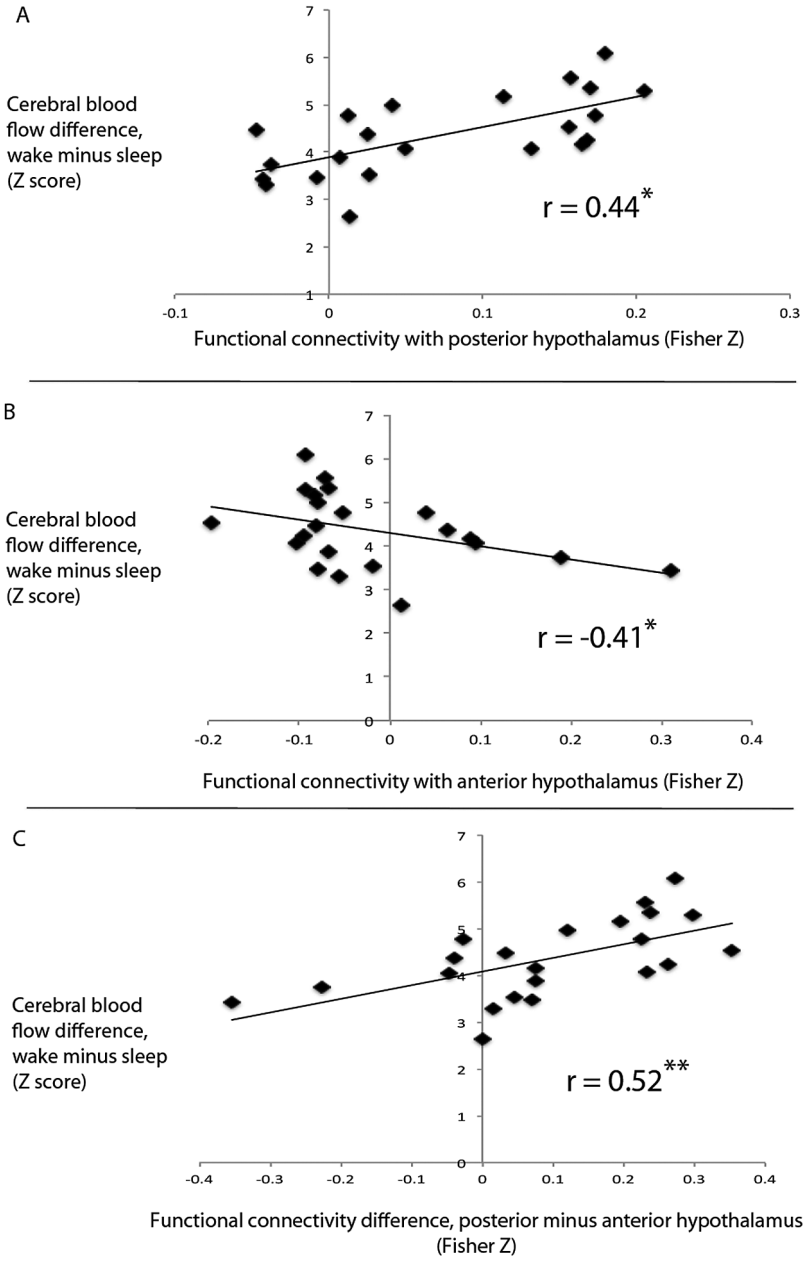

Figure 4. Functional connectivity with the hypothalamus predicts sleep–wake CBF change in other brain regions. Among regions with high contrast in CBF across sleep–wake states, the magnitude of the CBF change is correlated with functional connectivity to the posterior hypothalamus (A), the anterior hypothalamus (B), and especially the difference in connectivity between the posterior and anterior hypothalamus (C). *p < 0.05; **p < 0.01.

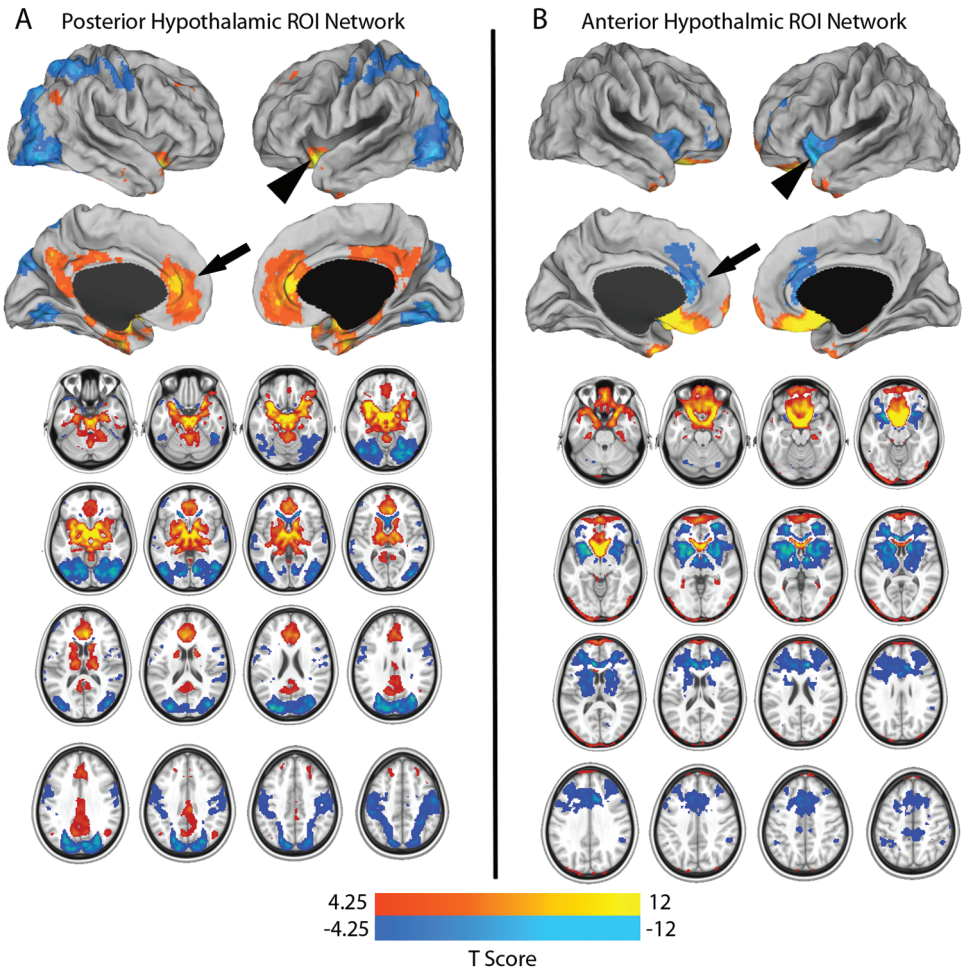

Figure 5. Resting-state functional connectivity with posterior (A) and anterior (B) regions of the hypothalamus. Positive correlations are shown in warm colors and negative correlations are in cool colors. Some areas show an inverse pattern of correlation with the two hypothalamic regions such as the ventral anterior insula (arrowhead) and anterior cingulate cortex (arrow). Axial slices range from Z = 90 to 240 spaced evenly every 10 mm, from left to right, in radiological orientation (right hemisphere on left). See Supplementary Figure S3 for similar results with alternate processing strategy and Supplementary Table S2 for MNI coordinates of regional peaks corresponding to this image.

Conjunction analysis

Results of the conjunction analysis are shown in Figure 6, with warm colors representing regions that are both positively correlated with the posterior hypothalamus and negatively correlated with the anterior hypothalamus, and cool colors representing the opposite relationship.

Discussion

In this article, we use rs-fcMRI to identify networks associated with regions of the hypothalamus implicated in promoting sleep (anterior hypothalamus ROI) and wakefulness (posterior hypothalamus ROI) based on homology with experimental animals. We show a negatively correlated pattern of BOLD activity between these hypothalamic regions. In addition, we relate these functional connectivity patterns to human brain regions modulated by sleep–wake based on previously published PET results. Although we view these findings cautiously until replicated by others, the results are provocative and warrant further investigation.

Is hypothalamic resting-state functional connectivity feasible?

The current study pushes the boundary of feasibility for resting-state functional connectivity, and results should be interpreted with caution until independently replicated. The hypothalamus is surrounded by potential sources of artifact for rs-fcMRI data including CSF pulsation, anatomic boundaries, blood vessels, and the optic nerve [66–68]. Furthermore, the spatial resolution of functional connectivity data (27 mm^3) is large relative to the size of our hypothalamic ROIs and the distance between our ROIs (9 mm). Spatial smoothing, which can help with registration errors across participants, further limits resolution [48–50]. Given these issues, there is good reason to be skeptical of the present results.

However, this is not the first study to use rs-fcMRI to investigate the hypothalamus [69, 70], and there are reasons to think that the present results may be valid. First, rs-fcMRI data with the same resolution and processing method can resolve adjacent thalamic nuclei that approach the size and spatial proximity of the hypothalamic nuclei in the present study [54, 62, 71]. Second, sensitivity to small nuclei may increase with the size of the rs-fcMRI cohort, and our N = 98 cohort has demonstrated unique connectivity patterns with small brainstem structures plagued by the same confounds as the hypothalamus [51, 53, 68]. Third, our results are largely independent of processing strategy. Although no strategy guarantees a clean hypothalamic signal, one would expect results due to artifact to vary more across different artifact reduction strategies. Fourth, limited spatial resolution should bias us against the present finding, increasing rather than decreasing

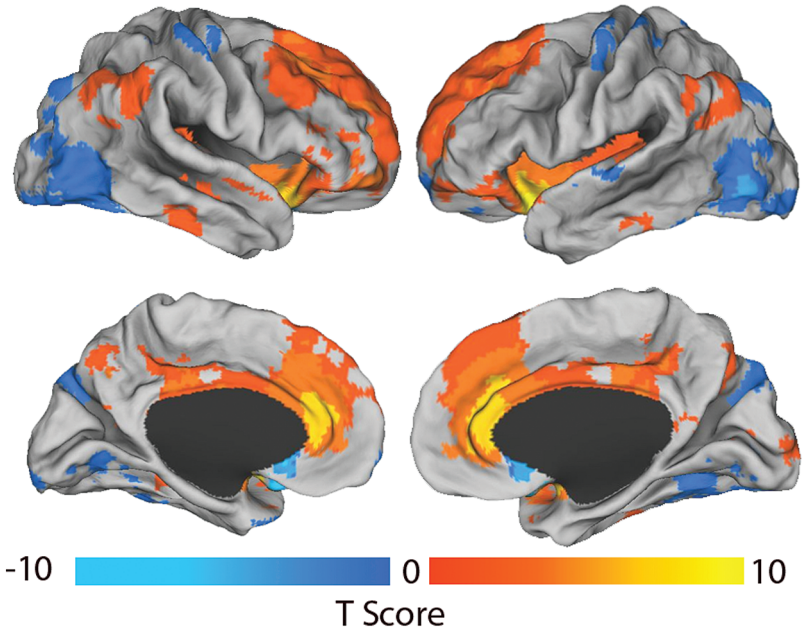

Figure 6. Intrinsically defined anticorrelated networks based on hypothalamic connectivity. Warm colors represent regions that have activity both positively correlated with the posterior hypothalamus as well as negatively correlated activity with the anterior hypothalamus. Cool colors represent regions that have both positively correlated activity with the anterior hypothalamus and negatively correlated activity with the posterior hypothalamus. Results are shown with varying statistical thresholds and preprocessing methods in Supplementary Figure S3.

the correlation between adjacent nuclei. Fifth, the results are spatially specific to the location of our a priori hypothalamic nuclei, not the expected location of artifact (e.g. along the brainstem for CSF pulsation, along a vessel for vascular pulsation) [54]. Finally, many of our rs-fcMRI results align with prior research using methods other than rs-fcMRI, as discussed below.

Network organization of sleep- and wake-promoting hypothalamic regions

Rs-fcMRI with the wake-promoting posterior hypothalamus and sleep-promoting anterior hypothalamus identified unique and complimentary brain networks. This represents a new approach to study sleep–wake circuitry that will require further validation. Most sleep–wake studies in both animals and humans have compared brain activity between states, measured during both wake and sleep. In contrast, the current study was based on spontaneous fluctuations of BOLD signal recorded predominately or exclusively within a state of quiet wakefulness, a within-state rather than between-state design. Although we cannot exclude the possibility that participants were falling asleep and waking up during collection of rs-fcMRI data, this seems unlikely given the frequency of the BOLD fluctuations. Participants would need to consistently fall asleep and wake up at a frequency of 0.009 to 0.08 Hz (approximately one sleep–wake cycle every 10–100 s) to generate the observed findings (Figure 2F). Instead, it is more likely that resting-state BOLD fluctuations in the hypothalamus are similar to resting-state BOLD fluctuations elsewhere in the brain and reflect ongoing spontaneous brain activity. The finding that regions routinely modulated in opposite directions by sleep and wake are anticorrelated at rest matches prior work showing that regions routinely modulated in opposite directions by tasks are anticorrelated at rest [44, 72].

The current network results appear to have some anatomical validity relative to known patterns of hypothalamic connectivity in experimental animals as well as regional anatomy related to human arousal. First, the anticorrelation between the anterior and posterior hypothalamus is consistent with inhibitory connections between these regions in experimental animals [29–31], and spatially specific to the most likely location of these nuclei in humans (Figure 2 and Supplementary Figure S1). Second, the posterior hypothalamus was functionally connected to other regions thought to have a role in arousal based on both animal and human research such as the upper brainstem, ventral anterior insula, and pregenual anterior cingulate [53, 73–79]. Third, our hypothalamic connectivity results align well with prior functional neuroimaging of sleep–wake states in humans. Specifically, regions previously shown to have the highest contrast in CBF between sleep–wake states were functionally connected to the wake-promoting posterior hypothalamus (and anticorrelated with the sleep-promoting anterior hypothalamus) [33].

The current study also identified regions correlated with the sleep-promoting anterior hypothalamus, raising the question whether these regions could be involved in promoting sleep. One such region is the subgenual cingulate/ventromedial prefrontal cortex whose possible role in sleep-promotion is supported by the following: (1) rat studies showing anatomical projections to the IN/VLPO and sleep fragmentation when this area is lesioned [32, 80], (2) cat studies showing induction of sleep when this area is stimulated [81], (3) monkey studies showing increased neuronal firing of this region during sleep [82], and (4) human studies showing elevated activity during deep sleep and periods of low arousal based on skin conductance [83, 84]. The extrastriate visual cortices also show this pattern and are relatively more active than other cortical areas during sleep [34, 63, 64, 83].

Interestingly, both the above patterns of hypothalamic connectivity were observed in different regions of the basal forebrain. The lateral basal forebrain was positively correlated with the posterior hypothalamic ROI and negatively correlated with

the anterior hypothalamic ROI, whereas the medial basal forebrain had the reverse pattern. The basal forebrain is typically thought of as part of the arousal circuitry; however, it contains several populations of neurons, some of which may be sleep-promoting [85, 86] or play a role in sleep–wake transitions [87]. Whether the posterolateral versus medial basal forebrain play different or opposing roles in sleep–wake regulation is a testable hypothesis for future work.

Functional significance of anticorrelated networks

The relationship between the networks derived from two functionally opposed regions of the hypothalamus is interesting in light of ongoing controversy surrounding the interpretation of rs-fcMRI anticorrelations [54–56]. The canonical example of anticorrelated networks is the relationship between the default mode network and the task-positive network, the latter of which encompasses the salience and dorsal attention networks [44, 88]. The inversely related activity between these networks was first appreciated from task-based functional imaging experiments [89], and it was subsequently discovered that these networks are intrinsically anticorrelated in the awake, resting brain, as indicated through rs-fcMRI [44, 90–92] and supported by electrocorticography [93–95].

Interpreting the functional significance of anticorrelation in default mode and task-positive networks has been challenging, in part because (1) the functions of these networks are complex and not unambiguously opposed, and (2) the anatomical connections mediating their anticorrelated relationship are unclear. The current results may contribute to our understanding of anticorrelations, as (1) there is robust evidence for functional antagonism between the hypothalamic regions investigated here with regard to sleep–wake states in experimental animals, and (2) there is a direct, inhibitory projection from the sleep-promoting hypothalamic region to the wake-promoting posterior hypothalamus, conserved across several species. As such, these results may align with prior animal work suggesting that rs-fcMRI anticorrelations can be detected between regions with direct inhibitory connections [96] as well as prior speculation that anticorrelations exist between functionally opposing networks [44, 97–99].

Limitations

There are several important limitations to the current analysis that warrant cautious interpretation of results until further replication is performed. First, although we often refer to our posterior and anterior hypothalamic ROIs as “wake-promoting” or “sleep-promoting” for convenience, these labels are based primarily on experiments in laboratory animals and presumed homology in humans. Whether these specific ROIs have the same functional role in sleep–wake regulation humans is unknown. Second, the spatial resolution of the imaging data is such that the hypothalamic ROIs likely mediate other hypothalamic functions not directly related to sleep–wake (e.g. thermoregulation and other aspects of homeostasis). Thus, activity related to these other functions may contribute to the network results reported here. Similarly there are other hypothalamic regions that also contribute to sleep–wake regulation that were not included here. Third, CSF, arterial, and venous flow around the hypothalamus introduces the possibility of non-neuronal contributions to the BOLD signal [66–68]. Although multiple preprocessing and analytic strategies were taken to circumvent these concerns, this remains a limitation of the current work. Relatedly, susceptibility artifact can occur in the ventromedial basal forebrain and prefrontal cortex and findings in this region must be considered preliminary until replicated using acquisition protocols optimized for these regions [100]. Fourth, we lack several variables that could further inform our results. We did not record EEG, skin conductance, or pupillometry while participants were in the scanner, and thus cannot exclude the possibility that some participants fell asleep or had large fluctuations in arousal or vigilance [87, 101–105]. We also do not know the sleep habits, blood pressure, or body mass of the participants. In addition, because the CBF data used in this analysis were from a separate cohort, we cannot verify the relationship of hypothalamic connectivity with CBF within the same cohort. Fifth, human neuroimaging studies including rs-fcMRI cannot speak to causality. Whether the hypothalamus drives sleep–wake changes observed in other brain regions and whether these regions are themselves involved in promoting wake versus sleep remains unknown. Finally, extrapolation of these results to other areas of inquiry, such as PET studies or animal neurophysiology, remains speculative and will require further investigation.

Future directions

This analysis raises several testable hypotheses. First, we predict that firing patterns of sleep- and wake-promoting hypothalamic regions will be inversely correlated within a given state if measured with simultaneous recordings in experimental animals. Second, we predict an inverse sleep–wake relationship in the medial versus lateral basal forebrain similar to the anterior versus posterior hypothalamus. Third, we hypothesize that the observed anticorrelation between the anterior and posterior hypothalamus and their associated networks will be disrupted in patients with sleep–wake dysregulation, such as narcolepsy or insomnia, consistent with the idea that competition between anticorrelated networks with opposing functions can optimize performance [44, 97–99].

Finally, we predict that the networks identified here can serve as useful targets for neuromodulation to promote sleep or wake. This idea of therapeutically modulating sleep–wake circuitry was presented in 1930 by Von Economo to conclude his seminal article localizing the sleep- and wake-promoting hypothalamic regions [1]. Advances in both invasive and noninvasive brain stimulation technology together with imaging insights into the regions and networks mediating sleep–wake regulation may soon allow us to realize Von Economo’s vision.

Supplementary Material

Supplementary material is available at *SLEEP* online.

Acknowledgments

We thank Randy Buckner and the Brain Genomics Superstruct Project for contributing data and analysis tools. A.D.B. designed the study, performed the analyses, and wrote the manuscript. D.F. helped to design and perform analyses. C.B.S. and J.C.G. helped to define the anatomical regions of interest. J.B. performed functional connectivity analyses. M.D.F. oversaw

all aspects of the project. All authors were involved in editing the manuscript and each contributed important ideas to the final product.

Funding

The work and investigators were supported by the following: Sidney R. Baer Jr. Foundation, NIH K12HD027748, K12NS098482, Fraternal Order of Eagles, and the Roy J. Carver Trust (A.D.B.), T32-HL007901-18, K08-NS099425 (J.C.G.), Howard Hughes Medical Institute, the Parkinson's Disease Foundation, the Mathers Foundation, K23NS083741 and R01 MH113929 (M.D.F.), and NIH RO1 NS085477 (C.B.S).

Notes

Conflict of interest statement. M.D.F. is listed as an inventor on submitted or issued patents on guiding neurological interventions with fMRI. A.D.B. serves as a consultant on a data safety and monitoring board for Ekso Bionics. The authors declare no competing financial interests.

References

1. Von Economo C. Sleep as a problem of localization. *J Nerv Ment Dis*. 1930;**71**:249–259.
2. Nauta WJ. Hypothalamic regulation of sleep in rats; an experimental study. *J Neurophysiol*. 1946;**9**:285–316.
3. Takahashi K, *et al*. Neuronal activity of histaminergic tuberomammillary neurons during wake-sleep states in the mouse. *J Neurosci*. 2006;**26**(40):10292–10298.
4. Gerashchenko D, *et al*. Effects of lateral hypothalamic lesion with the neurotoxin hypocretin-2-saporin on sleep in Long-Evans rats. *Neuroscience*. 2003;**116**(1):223–235.
5. Swett CP, *et al*. The effects of posterior hypothalamic lesions on behavioral and electrographic manifestations of sleep and waking in cats. *Arch Ital Biol*. 1968;**106**(3):283–293.
6. Yu X, *et al*. Wakefulness is governed by GABA and histamine cotransmission. *Neuron*. 2015;**87**(1):164–178.
7. Lin JS, *et al*. The waking brain: an update. *Cell Mol Life Sci*. 2011;**68**(15):2499–2512.
8. Anaclet C, *et al*. Orexin/hypocretin and histamine: distinct roles in the control of wakefulness demonstrated using knock-out mouse models. *J Neurosci*. 2009;**29**(46):14423–14438.
9. Lin JS, *et al*. A critical role of the posterior hypothalamus in the mechanisms of wakefulness determined by microinjection of muscimol in freely moving cats. *Brain Res*. 1989;**479**(2):225–240.
10. Pedersen NP, *et al*. Supramammillary glutamate neurons are a key node of the arousal system. *Nat Commun*. 2017;**8**(1):1405.
11. Panula P, *et al*. Histamine-immunoreactive nerve fibers in the rat brain. *Neuroscience*. 1989;**28**(3):585–610.
12. Lin JS, *et al*. Histaminergic descending inputs to the mesopontine tegmentum and their role in the control of cortical activation and wakefulness in the cat. *J Neurosci*. 1996;**16**(4):1523–1537.
13. Hong EY, *et al*. Retrograde study of projections from the tuberomammillary nucleus to the mesopontine cholinergic complex in the rat. *Brain Res*. 2011;**1383**:169–178.
14. Lu J, *et al*. Effect of lesions of the ventrolateral preoptic nucleus on NREM and REM sleep. *J Neurosci*. 2000;**20**(10):3830–3842.
15. Sherin JE, *et al*. Activation of ventrolateral preoptic neurons during sleep. *Science*. 1996;**271**(5246):216–219.
16. Szymusiak R, *et al*. Sleep-related neuronal discharge in the basal forebrain of cats. *Brain Res*. 1986;**370**(1):82–92.
17. Suntsova N, *et al*. Sleep-waking discharge patterns of median preoptic nucleus neurons in rats. *J Physiol*. 2002;**543**(Pt 2):665–677.
18. McGinty DJ, *et al*. Sleep suppression after basal forebrain lesions in the cat. *Science*. 1968;**160**(3833):1253–1255.
19. Sherin JE, *et al*. Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. *J Neurosci*. 1998;**18**(12):4705–4721.
20. Takahashi K, *et al*. Characterization and mapping of sleep-waking specific neurons in the basal forebrain and preoptic hypothalamus in mice. *Neuroscience*. 2009;**161**(1):269–292.
21. Sakai K, *et al*. Sleep-waking discharge of ventral tuberomammillary neurons in wild-type and histidine decarboxylase knock-out mice. *Front Behav Neurosci*. 2010;**4**:53.
22. Steininger TL, *et al*. Sleep-waking discharge of neurons in the posterior lateral hypothalamus of the albino rat. *Brain Res*. 1999;**840**(1-2):138–147.
23. Szymusiak R, *et al*. Preoptic area sleep-regulating mechanisms. *Arch Ital Biol*. 2001;**139**(1-2):77–92.
24. Szymusiak R, *et al*. Hypothalamic regulation of sleep and arousal. *Ann N Y Acad Sci*. 2008;**1129**:275–286.
25. McGinty D, *et al*. Sleep-promoting functions of the hypothalamic median preoptic nucleus: inhibition of arousal systems. *Arch Ital Biol*. 2004;**142**(4):501–509.
26. Saper CB, *et al*. Hypothalamic regulation of sleep and circadian rhythms. *Nature*. 2005;**437**(7063):1257–1263.
27. Liu YW, *et al*. Histamine regulates activities of neurons in the ventrolateral preoptic nucleus. *J Physiol*. 2010;**588**(Pt 21):4103–4116.
28. Steininger TL, *et al*. Subregional organization of preoptic area/anterior hypothalamic projections to arousal-related monoaminergic cell groups. *J Comp Neurol*. 2001;**429**(4):638–653.
29. Gallopin T, *et al*. Identification of sleep-promoting neurons in vitro. *Nature*. 2000;**404**(6781):992–995.
30. Manns ID, *et al*. Alpha 2 adrenergic receptors on GABAergic, putative sleep-promoting basal forebrain neurons. *Eur J Neurosci*. 2003;**18**(3):723–727.
31. Lin JS, *et al*. Hypothalamo-preoptic histaminergic projections in sleep-wake control in the cat. *Eur J Neurosci*. 1994;**6**(4):618–625.
32. Chou TC, *et al*. Afferents to the ventrolateral preoptic nucleus. *J Neurosci*. 2002;**22**(3):977–990.
33. Braun AR, *et al*. Regional cerebral blood flow throughout the sleep-wake cycle. An H2(15)O PET study. *Brain*. 1997;**120** (Pt 7):1173–1197.
34. Jakobson AJ. Brain activity in sleep compared to wakefulness: a meta-analysis. *J Behav Brain Sci*. 2012;**2**(2):249–257.
35. Kaufmann C, *et al*. Brain activation and hypothalamic functional connectivity during human non-rapid eye movement sleep: an EEG/fMRI study. *Brain*. 2006;**129**(Pt 3):655–667.
36. Nofzinger EA, *et al*. Human regional cerebral glucose metabolism during non-rapid eye movement sleep in relation to waking. *Brain*. 2002;**125**(Pt 5):1105–1115.

37. Maquet P. Functional neuroimaging of normal human sleep by positron emission tomography. *J Sleep Res*. 2000;**9**(3):207–231.
38. Fonov V, *e al*. Unbiased nonlinear average age-appropriate brain templates from birth to adulthood. *Neuroimage*. 2009;**47**:S102.
39. Baroncini M, *et al*. MRI atlas of the human hypothalamus. *Neuroimage*. 2012;**59**(1):168–180.
40. Krolewski DM, *et al*. Expression patterns of corticotropin-releasing factor, arginine vasopressin, histidine decarboxylase, melanin-concentrating hormone, and orexin genes in the human hypothalamus. *J Comp Neurol*. 2010;**518**(22):4591–4611.
41. Gai WP, *et al*. Galanin immunoreactive neurons in the human hypothalamus: colocalization with vasopressin-containing neurons. *J Comp Neurol*. 1990;**298**(3):265–280.
42. Lin JS. Brain structures and mechanisms involved in the control of cortical activation and wakefulness, with emphasis on the posterior hypothalamus and histaminergic neurons. *Sleep Med Rev*. 2000;**4**(5):471–503.
43. Holmes AJ, *et al*. Brain genomics superstruct project initial data release with structural, functional, and behavioral measures. *Sci Data*. 2015;**2**:150031.
44. Fox MD, *et al*. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc Natl Acad Sci U S A*. 2005;**102**(27):9673–9678.
45. Van Dijk KR, *et al*. Intrinsic functional connectivity as a tool for human connectomics: theory, properties, and optimization. *J Neurophysiol*. 2010;**103**(1):297–321.
46. Fox MD, *et al*. Efficacy of transcranial magnetic stimulation targets for depression is related to intrinsic functional connectivity with the subgenual cingulate. *Biol Psychiatry*. 2012;**72**(7):595–603.
47. Fox MD, *et al*. Measuring and manipulating brain connectivity with resting state functional connectivity magnetic resonance imaging (fcMRI) and transcranial magnetic stimulation (TMS). *Neuroimage*. 2012;**62**(4):2232–2243.
48. Scouten A, *et al*. Spatial resolution, signal-to-noise ratio, and smoothing in multi-subject functional MRI studies. *Neuroimage*. 2006;**30**(3):787–793.
49. Pajula J, *et al*. Effects of spatial smoothing on inter-subject correlation based analysis of FMRI. *Magn Reson Imaging*. 2014;**32**(9):1114–1124.
50. Mikl M, *et al*. Effects of spatial smoothing on fMRI group inferences. *Magn Reson Imaging*. 2008;**26**(4):490–503.
51. Boes AD, *et al*. Network localization of neurological symptoms from focal brain lesions. *Brain*. 2015;**138**(Pt 10):3061–3075.
52. Laganiere S, *et al*. Network localization of hemichorea-hemiballismus. *Neurology*. 2016;**86**(23):2187–2195.
53. Fischer DB, *et al*. A human brain network derived from coma-causing brainstem lesions. *Neurology*. 2016;**87**(23):2427–2434.
54. Fox MD, *et al*. The global signal and observed anticorrelated resting state brain networks. *J Neurophysiol*. 2009;**101**(6):3270–3283.
55. Murphy K, *et al*. Towards a consensus regarding global signal regression for resting state functional connectivity MRI. *Neuroimage*. 2017;**154**:169–173.
56. Murphy K, *et al*. The impact of global signal regression on resting state correlations: are anti-correlated networks introduced? *Neuroimage*. 2009;**44**(3):893–905.
57. Behzadi Y, *et al*. A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. *Neuroimage*. 2007;**37**(1):90–101.
58. Whitfield-Gabrieli S, *et al*. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connect*. 2012;**2**(3):125–141.
59. Di Martino A, *et al*. Functional connectivity of human striatum: a resting state FMRI study. *Cereb Cortex*. 2008;**18**(12):2735–2747.
60. Margulies DS, *et al*. Mapping the functional connectivity of anterior cingulate cortex. *Neuroimage*. 2007;**37**(2):579–588.
61. Marrelec G, *et al*. Partial correlation for functional brain interactivity investigation in functional MRI. *Neuroimage*. 2006;**32**(1):228–237.
62. Zhang D, *et al*. Noninvasive functional and structural connectivity mapping of the human thalamocortical system. *Cereb Cortex*. 2010;**20**(5):1187–1194.
63. Hofle N, *et al*. Regional cerebral blood flow changes as a function of delta and spindle activity during slow wave sleep in humans. *J Neurosci*. 1997;**17**(12):4800–4808.
64. Kjaer TW, *et al*. Regional cerebral blood flow during light sleep–a H(2)(15)O-PET study. *J Sleep Res*. 2002;**11**(3):201–207.
65. Maquet P, *et al*. Functional neuroanatomy of human slow wave sleep. *J Neurosci*. 1997;**17**(8):2807–2812.
66. Laufs H, *et al*. 'Brain activation and hypothalamic functional connectivity during human non-rapid eye movement sleep: an EEG/fMRI study'–its limitations and an alternative approach. *Brain*. 2007;**130**(Pt 7):e75; author reply e76.
67. Beissner F, *et al*. Advances in functional magnetic resonance imaging of the human brainstem. *Neuroimage*. 2014;**86**:91–98.
68. Harvey AK, *et al*. Brainstem functional magnetic resonance imaging: disentangling signal from physiological noise. *J Magn Reson Imaging*. 2008;**28**(6):1337–1344.
69. Wright H, *et al*. Differential effects of hunger and satiety on insular cortex and hypothalamic functional connectivity. *Eur J Neurosci*. 2016;**43**(9):1181–1189.
70. Kullmann S, *et al*. Resting-state functional connectivity of the human hypothalamus. *Hum Brain Mapp*. 2014;**35**(12):6088–6096.
71. Zhang D, *et al*. Intrinsic functional relations between human cerebral cortex and thalamus. *J Neurophysiol*. 2008;**100**:1740–1748.
72. Tavor I, *et al*. Task-free MRI predicts individual differences in brain activity during task performance. *Science*. 2016;**352**(6282):216–220.
73. Saper CB. Reciprocal parabrachial-cortical connections in the rat. *Brain Res*. 1982;**242**(1):33–40.
74. Hurley KM, *et al*. Efferent projections of the infralimbic cortex of the rat. *J Comp Neurol*. 1991;**308**(2):249–276.
75. Aston-Jones G, *et al*. Adaptive gain and the role of the locus coeruleus-norepinephrine system in optimal performance. *J Comp Neurol*. 2005;**493**(1):99–110.
76. Coste CP, *et al*. Cingulo-opercular network activity maintains alertness. *Neuroimage*. 2016;**128**:264–272.
77. Sadaghiani S, *et al*. Intrinsic connectivity networks, alpha oscillations, and tonic alertness: a simultaneous electroencephalography/functional magnetic resonance imaging study. *J Neurosci*. 2010;**30**(30):10243–10250.
78. Parvizi J, *et al*. Neuroanatomical correlates of brainstem coma. *Brain*. 2003;**126**(Pt 7):1524–1536.
79. Fuller PM, *et al*. Reassessment of the structural basis of the ascending arousal system. *J Comp Neurol*. 2011;**519**(5):933–956.

80. Chang CH, *et al*. Ventromedial prefrontal cortex regulates depressive-like behavior and rapid eye movement sleep in the rat. *Neuropharmacology*. 2014;**86**:125–132.
81. Alnaes E, *et al*. EEG synchronization and sleep induced by stimulation of the medial and orbital frontal cortex in cat. *Acta Physiol Scand*. 1973;**87**(1):96–104.
82. Rolls ET, *et al*. Activity of primate subgenual cingulate cortex neurons is related to sleep. *J Neurophysiol*. 2003;**90**:134–142.
83. Nofzinger EA, *et al*. Changes in forebrain function from waking to REM sleep in depression: preliminary analyses of [18F]FDG PET studies. *Psychiatry Res*. 1999;**91**(2):59–78.
84. Nagai Y, *et al*. Activity in ventromedial prefrontal cortex covaries with sympathetic skin conductance level: a physiological account of a "default mode" of brain function. *Neuroimage*. 2004;**22**(1):243–251.
85. Xu M, *et al*. Basal forebrain circuit for sleep-wake control. *Nat Neurosci*. 2015;**18**(11):1641–1647.
86. Anaclet C, *et al*. Basal forebrain control of wakefulness and cortical rhythms. *Nat Commun*. 2015;**6**:8744.
87. Lee SH, *et al*. Neuromodulation of brain states. *Neuron*. 2012;**76**(1):209–222.
88. Seeley WW, *et al*. Dissociable intrinsic connectivity networks for salience processing and executive control. *J Neurosci*. 2007;**27**(9):2349–2356.
89. Raichle ME, *et al*. A default mode of brain function. *Proc Natl Acad Sci U S A*. 2001;**98**(2):676–682.
90. Fransson P. Spontaneous low-frequency BOLD signal fluctuations: an fMRI investigation of the resting-state default mode of brain function hypothesis. *Hum Brain Mapp*. 2005;**26**(1):15–29.
91. Greicius MD, *et al*. Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. *Proc Natl Acad Sci U S A*. 2004;**101**(13):4637–4642.
92. Chai XJ, *et al*. Selective development of anticorrelated networks in the intrinsic functional organization of the human brain. *J Cogn Neurosci*. 2014;**26**(3):501–513.
93. Popa D, *et al*. Contrasting activity profile of two distributed cortical networks as a function of attentional demands. *J Neurosci*. 2009;**29**(4):1191–1201.
94. Keller CJ, *et al*. Neurophysiological investigation of spontaneous correlated and anticorrelated fluctuations of the BOLD signal. *J Neurosci*. 2013;**33**(15):6333–6342.
95. He BJ, *et al*. Electrophysiological correlates of the brain's intrinsic large-scale functional architecture. *Proc Natl Acad Sci U S A*. 2008;**105**(41):16039–16044.
96. Liang Z, *et al*. Anticorrelated resting-state functional connectivity in awake rat brain. *Neuroimage*. 2012;**59**(2):1190–1199.
97. Buckner RL, *et al*. The brain's default network: anatomy, function, and relevance to disease. *Ann N Y Acad Sci*. 2008;**1124**:1–38.
98. Kelly AM, *et al*. Competition between functional brain networks mediates behavioral variability. *Neuroimage*. 2008;**39**(1):527–537.
99. Hampson M, *et al*. Functional connectivity between task-positive and task-negative brain areas and its relation to working memory performance. *Magn Reson Imaging*. 2010;**28**(8):1051–1057.
100. Kundu P, *et al*. Multi-echo fMRI: a review of applications in fMRI denoising and analysis of BOLD signals. *Neuroimage*. 2017;**154**(March):59–80.
101. Kinomura S, *et al*. Activation by attention of the human reticular formation and thalamic intralaminar nuclei. *Science*. 1996;**271**(5248):512–515.
102. Paus T, *et al*. Time-related changes in neural systems underlying attention and arousal during the performance of an auditory vigilance task. *J Cogn Neurosci*. 1997;**9**(3):392–408.
103. Tagliazucchi E, *et al*. Decoding wakefulness levels from typical fMRI resting-state data reveals reliable drifts between wakefulness and sleep. *Neuron*. 2014;**82**(3):695–708.
104. Horovitz SG, *et al*. Decoupling of the brain's default mode network during deep sleep. *Proc Natl Acad Sci U S A*. 2009;**106**(27):11376–11381.
105. Picchioni D, *et al*. Decreased connectivity between the thalamus and the neocortex during human nonrapid eye movement sleep. *Sleep*. 2014;**37**(2):387–397.