RESTING-STATE FUNCTIONAL MAGNETIC RESONANCE IMAGING

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Sleep and resting-state functional MRI connectivity in middle-aged adults and elderly: A population-based study. Journal of Sleep Research 2020

ABSTRACT

Sleep problems increase with aging. Increasing evidence suggest that sleep problems are not only a consequence of the aging process, but may independently contribute to developing vascular or neurodegenerative brain disease. Yet, it remains unclear what mechanisms underlie the impact sleep problems may have on brain health in the general middle-aged and elderly population.

Here, we studied sleep's relation to specifically brain functioning in 621 participants (median age 62 years, 55% women) from the population-based Rotterdam Study. We investigated cross-sectional associations of polysomnographic and subjectively measured aspects of sleep with intrinsic neural activity measured with resting-state functional magnetic resonance imaging on a different day. We investigated both functional connectivity between regions and brain activity (blood-oxygen-level-dependent signal amplitude) within regions, hierarchically towards smaller topographical levels.

We found that longer polysomnographic total sleep time is associated with lower blood-oxygen-level-dependent signal amplitude in (pre)frontal regions. No objective or subjective sleep parameters were associated with functional connectivity between or within resting-state networks.

Findings may indicate a pathway through which sleep, in a 'real-life' population setting, impacts brain activity or regional brain activity determines of total sleep time.

INTRODUCTION

Sleep is a homeostatic process serving vital functions for the brain to support performance the next day. As adults age, they increasingly experience sleep problems.[1] Sleep problems have been hypothesized to impair brain health, as they are associated with developing stroke[2] and dementia.[3] It is therefore important that we increase our understanding how sleep, beyond its homeostatic, night-to-day effect, may impact brain health in the general middle-aged and elderly population.

How sleep affects the brain can be investigated well by studying brain functional connectivity. Brain functional connectivity can be studied non-invasively with functional MRI (fMRI), which measures intrinsic neural activity indirectly through blood oxygenation. Applying fMRI when individuals are not engaged in a task ('resting-state' fMRI (rs-fMRI)) reveals how brain regions spontaneously communicate with each other in connected networks.[4] Intrinsic neural activity as measured with rs-fMRI can provide measures of activity between cortical regions, or within them. The organization of intrinsic neural activity in networks is remarkably robust and present across various conditions.[5]

That sleep is relevant for waking rs-fMRI neural activity has been shown using various approaches. Experimental sleep deprivation studies showed immediate widespread changes in functional connectivity during subsequent wakefulness[6,7] including an increase of global fMRI-signal variability,[8] also known as signal amplitude. Importantly, observational studies that associated habitual sleep quality or duration, or a sleep disorder such as insomnia with rs-fMRI measures suggest that sleep may impact intrinsic neural activity beyond the short term.[9-14] Yet, only few studies measured sleep objectively to minimize misclassification or used large samples to increase statistical power and decrease the chance that significant associations are overestimated. Findings from large-scale, population-based studies are more equivocal, reporting no associations of sleep quality with connectivity between networks[15] or of self-reported sleep duration with signal amplitude in the often-studied 'default mode' network.[12]

It is therefore unclear if variations in sleep, including total sleep time and duration of individual sleep stages, are related to intrinsic neural activity during daytime, measured as functional connectivity between or neural activity within different brain regions, in the general middle-aged and elderly population. We aimed to fill this knowledge gap using sleep parameters measured with polysomnography and the Pittsburgh Sleep Quality Index, and rs-fMRI measures from the population-based Rotterdam Study cohort. We explored associations between sleep and intrinsic neural activity using a hierarchical approach from global to more spatially-specific analyses, and subsequently examined associations of total sleep time more regionally based on initial findings.

METHODS

Study setting

The Rotterdam Study, starting in 1990, is a prospective population-based cohort of inhabitants of a suburban district in Rotterdam aged 45 years or over.[16] Participating inhabitants were interviewed at home and subsequently visited the research center. These examination rounds were repeated every 4-5 years. The cohort was expanded twice, in 2000 with persons aged ≤55, and in 2006 with persons aged ≤45. We studied individuals from all three inclusion rounds who participated in a polysomnography (PSG) study between January 2012 to September 2014, and also underwent a resting state fMRI (rs-fMRI) scan. Rs-fMRI was implemented routinely since 2012.[17]

The Rotterdam Study (RS) has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The RS has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl) and into the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/network/primary/en/) under shared catalogue number NTR6831. The study was conducted in accordance with the guideline proposed in the World Medical Association Declaration of Helsinki. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

Study sample

We invited 1,750 persons that visited the research center for in-home PSG; 928 consented. Invitees were deemed able to understand study purpose and instructions. Twenty-seven recordings failed or were of insufficient quality for sleep scoring. Of these, 724 persons without MRI contra-indications also underwent rs-fMRI. We excluded participants with poor quality[17] rs-fMRI data (n=49), cortical brain infarcts (n=20) or with prevalent dementia or missing dementia screening (n=2). From the remaining 653, we included in our main analyses 621 participants with a time interval between PSG and rs-fMRI of one year or less. From this population, we included 560 participants for analyses of PSG spectral power due to failure of critical EEG-leads in 61 individuals. Similarly, we included 603 participants for analyses on global PSQI score due to missing data of more than one component (see Sleep assessments below).

Sleep assessments

We recorded one night of PSG at home during weeknights. Polysomnography was applied by trained research assistants according to the American Association of Sleep Medicine (AASM) criteria,[18] including six electroencephalography (EEG) channels (F3/A2,

F4/A1, C3/A2, C4/A1, O1/A2, O2/A1), bilateral electro-oculography, chin electromyography, electrocardiography, respiratory belts on the chest and abdomen, oximetry, and a nasal pressure transducer and oronasal thermocouple. Participants were instructed to spend the night as normal as possible, without restrictions on bedtimes and use of alcohol, coffee or sleep medication. They pressed a button to signal when intending to go to sleep ("lights out") and getting out of bed ("lights on").

Sleep was scored[18] by a Registered Polysomnographic Technologist to determine total sleep time (TST), sleep onset latency (SOL), wake after sleep onset (WASO), sleep efficiency (SE), and the duration of the sleep stages non-rapid eye movement (NREM) 1 (N1), N2, N3 and REM.

We calculated spectral power and spindles in the C3/A2 derivation, using PRANA software (PhiTools, Strasbourg, France). For spectral power, band-pass filtering (0.125-128 Hz) and automated removal of artifacts were applied. Spectral analysis was performed using 4-second epochs with 50% overlap, averaged over 30-second epochs. We calculated the absolute spectral power in the delta (0.75-4.00 Hz), beta (15.50-22.50 Hz) and gamma (22.50-40.00 Hz) frequency bands.

Apneas were defined as an airflow reduction of ≥90% of baseline for ≥10 seconds, and a hypopnea was defined as an airflow reduction of ≥30% of baseline for ≥10 seconds and a desaturation of ≥3% from baseline or an arousal.[18] The apnea-hypopnea index (AHI) was automatically calculated as the number of apneas and hypopneas per hour of sleep.

Subjective sleep quality during the past 4 weeks was measured with the PSQI during the home interview. The PSQI has good test-retest reliability and validity in a non-clinical sample of older adults.[19] Items, including self-reported sleep duration, were scored to provide a global PSQI score ranging from 0-21. Higher scores indicate poorer sleep quality. We weighted the PSQI score for 36 out of 603 individuals with one component score missing, by multiplying scores by 7/6.

To validate our findings for polysomnography sleep measure and assess a possible first night effect we used actigraphy.[20] The night of polysomnography, participants also wore an actigraph (ActiWatch model AW4, Cambridge Technology Ltd), and were invited to wear it for 7 days and also keep a sleep diary. Of 621 participants, 428 completed at least 4 consecutive nights (recording duration: 153±16 hours [median=144]). We used diary-derived times of 'lights out' and getting up the next morning to estimate time in bed. Within the time in bed, total sleep time was estimated using a validated algorithm with a threshold of 20 activity counts, and was averaged over all available nights per participant to estimate habitual total sleep time.

Neuroimaging

Brain imaging was performed with a 1.5-tesla MRI scanner (Signa Excite II, GE Healthcare, Milwaukee, WI, USA) at the research center. Resting state fMRI acquisition time was 7m44s (TR= 2900 ms, TE= 60ms, Field of View= 21 cm^2, 31 axial slices, matrix size=64x64, slice thickness= 3.3 mm, 165 volumes). Details of rs-fMRI preprocessing and connectivity analyses are provided elsewhere.[17] In brief, participants were prompted before the start of the fMRI-sequence to lie still, keep their eyes open, and stay awake. Preprocessing of resting-state data was performed with the FMRIB Software Library FEAT package.[21] Subject-specific artifact removal was conducted using independent components which were automatically classified. We excluded scans that showing absolute head displacement >3 mm and/or mean relative frame-wise displacement >0.2 mm. Also, as mild ghosting artefacts were introduced during rs-fMRI acquisition, we did not include scans with a ghost-to-signal ratio>0.1 and added this ratio as a covariate in analyses.[17]

For functional connectivity analyses, we generated a study-specific functional parcellation using independent component analysis[17,22] resulting in 50 components of interest, or functional nodes (hereafter: nodes). A node thus is a region where voxels show the same temporal BOLD-signal pattern. This template was used to derive node-level time series and obtain values for the full temporal correlations per subject for all nodes. Using hierarchical clustering of the group-level node correlations,[22] we concatenated these nodes into 9 large-scale networks, labeled anterior default mode, posterior default mode, frontoparietal, dorsal attention, ventral attention, sensorimotor, visual, subcortical, and temporal network.[17] Networks thus contain multiple nodes showing similar temporal patterns. Defining small nodes and clustering them into networks allowed studying with more detail the functional specialization within networks, as well as large-scale networks as a whole.[23]

Using the functional parcellation of 50 nodes, we calculated functional connectivity between node regions, and brain activity within node regions. For functional connectivity, we calculated correlations between the BOLD-signal time-series of each of the 50 nodes with all others. At the network level, we obtained between-network functional connectivity by averaging correlation values between all nodes from one network with all nodes from the other network, for 9x9 networks. Within-network functional connectivity was thus defined by averaging correlations of node pairs within that network. We investigated brain activity within regions as the variability of that region's BOLD-signal, by calculating the standard deviation (SD) of each node's time series (hereafter: signal amplitude). Analogous to functional connectivity, network-level signal amplitude was obtained by averaging amplitudes across nodes within that network. Global signal amplitude was obtained by averaging over all 50 nodes.

Potential confounders

We adjusted for potential confounders selected based on relevant publications[17,24]: Age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume.

The sensitivity analysis included additionally adjusting the main analyses for depressive symptoms and use of any antidepressant or hypnotic medication during PSG. Details of measurement are provided in the Supplementary Text.

Statistical analyses

Details are described in the Supplementary Text. We investigated cross-sectional associations of 12 sleep determinants (TST, WASO, SOL, SE, duration of stages N1, N2, N3, REM, spectral delta, beta and gamma power, and global PSQI score) with both functional connectivity between regions (and within where possible), and signal amplitude within regions. We used non-parametric permutation testing (n=5,000) implemented in FSL's 'randomise', with family-wise error (FWE) corrected P-values.

We hierarchically tested associations to examine regional heterogeneity if significant at a global level: We investigated associations with functional connectivity at the network level, and further analyzed node-level associations if nominally significant. Similarly, we first investigated associations with mean signal amplitude on a global level, and further analyzed the nominally significant associations on a network level. Furthermore, we investigated significant network-level associations on a node level.

As tests in 'randomise' are by default performed one-sided, we further Bonferroni-corrected the alpha level of 0.05 to $P_{FWE\text{-}corrected}<0.025$ (nominal significance level). As we tested multiple sleep determinants, we defined a more stringent threshold for significance at $P_{FWE\text{-}corrected}<0.00277$ (number of effective independent tests=9.23).

As sensitivity analysis, we repeated the analyses in persons with a shorter time interval between imaging and sleep measurements (<1 month for PSG parameters; <6 months for PSQI score). Also, we additionally adjusted analyses for i) depressive symptoms and use of any antidepressant or hypnotic medication during PSG; ii) AHI.

In post-hoc analyses based on initial findings for total sleep time, we i) explored associations of separate sleep stages with amplitude on a node level; ii) assessed possible non-linearity by analyzing 5 equal-sized categories (quintiles) of total sleep time and modeling a quadratic term; iii) repeated analyses with actigraphy-estimated total sleep time in n=428 with valid actigraphy data, and with self-reported sleep duration assessed in the PSQI.

RESULTS

We included 621 participants (median age=62 years [range 52-95 years], 55% women). The median absolute time interval between PSG and rs-fMRI was 17 days. Excluded participants did not differ by age, sex, head motion parameters or sleep stages duration from included participants. Correlations amongst sleep and fMRI parameters are provided in Supplementary Table 1.

We found no associations of objective or subjective sleep parameters with functional connectivity between or within resting state networks (all $P_{FWE\text{-}corrected}$ >0.025; Fig. 1).

We observed an association of longer total sleep time with lower mean global signal amplitude (beta per SD increase: -0.025 (95% CI -0.044; -0.006); P=5.0*e*-3; Table 2).

Investigating the regional heterogeneity of this association at a network level, we found it was present in the ventral attention, sensorimotor, subcortical, and temporal network (Table 3). In the ventral attention network, the association remained after correcting for testing multiple sleep parameters (-0.051 (95% CI -0.077; -0.024); $P_{FWE\text{-}corrected}$=1.2*e*-3; Supplementary Figure 1).

We further investigated associations of total sleep time with signal amplitude within aforementioned networks at the node level. We only observed associations of longer total sleep time with lower signal amplitude in nodes of the ventral attention network, distributed mainly in (pre)frontal regions (Fig. 2). The association in 'node 32' remained after correcting for multiple testing (-0.051 (95% CI -0.075;-0.027); $P_{FWE\text{-}corrected}$=1.6*e*-3). This node corresponds bilaterally to the anterior cingulate gyrus, and the juxtapositional lobule cortex (formerly: Supplementary motor cortex; Fig. 2).

Other sleep parameters were not associated with mean global signal amplitude, yet direction of effect sizes were mostly congruent with indicating 'poor' sleep (e.g. sleep onset latency, beta spectral power) versus 'good' sleep (e.g. sleep efficiency).

Restricting associations to persons with a shorter time interval between sleep and rs-fMRI measurement showed more pronounced effect sizes for the association of longer total sleep time with lower mean signal amplitude (n=450, Supplementary Table 2). Associations remained statistically significant in 'node 32' (-0.063 (95% CI -0.091; -0.034); $P_{FWE\text{-}corrected}$=1.0*e*-3), and 'node 23' (-0.080 (95% CI -0.120; -0.040); $P_{FWE\text{-}corrected}$=2.0*e*-3) corresponding mainly to the frontal pole and the anterior cingulate gyrus (Fig. 2). Longer stage N2 sleep related with lower global mean signal amplitude, driven mostly by the ventral attention and temporal networks (Supplementary Table 2), yet no node-level associations survived multiple testing correction.

In the total sample of n=621, additional adjustment for depressive symptoms and use of antidepressant and hypnotic medication during PSG did not change estimates on the global level (-0.025 (95% CI 0.044;-0.006); P=5.0*e*-3), or network level (ventral attention network: -0.051 (95% CI -0.078; -0.025); $P_{FWE\text{-}corrected}$=1.2*e*-3; other networks: all

Table 1. Characteristics of the study population

Characteristics (unit)	Value
Age (years)	62 (58; 66)
Female	340 (55%)
Time interval MRI-PSG (days)	6 (-12; 22)
No. of participants <1 month	450 (72%)
Time interval MRI-PSQI (days)	150 (104; 191)
No. of participants <6 months	438 (69%)
Habitual alcohol consumption (gr/day)	8 (4; 11)
Physical activity (MET-hours/week)	50 (24; 78)
Systolic blood pressure (mm Hg)	133 ± 18
Body mass index (kg/m^2)	27 ± 4
History of diabetes mellitus	73 (12%)
Supratentorial gray matter volume (cm^3)	538 ± 55
Intracranial volume (cm^3)	1,141 ± 115
Depressive symptoms (CES-D score)	12 (10; 15)
Use antidepressants/hypnotics during PSG	29 (5%)
Self-reported sleep duration (minutes)	408 ± 73
Apnea-hypopnea index (events/hour of sleep)	9 (5; 13)
Sleep parameters	
Total sleep time (minutes)	380 ± 65
Sleep onset latency (minutes)	14 (8; 23)
Wake after sleep onset (minutes)	71 ± 48
Sleep efficiency (%)	81% ± 11
Sleep stage duration (minutes)	
N1	49 ± 25
N2	203 ± 52
N3	48 ± 37
REM	79 ± 26
Absolute spectral power (μV^2/Hz)	
Delta (range: 0.75 - 4.50 Hz)	106 (72; 155)
Beta (range: 15.50 - 22.50 Hz)	2.5 (1.7; 3.7)
Gamma (range: 22.50 – 40.00 Hz)	1.9 (1.3; 2.9)
Missing	61 (10%)
Subjective sleep quality (PSQI score)	3 (1; 6)
Missing	18 (3%)

Values are frequency (%) for categorical variables, and mean ± standard deviation or median (1st quartile; 3rd quartile) for continuous variables, calculated over 621 participants unless specified otherwise. Values include imputed values for covariates.

Abbreviations: CES-D=Center for Epidemiological Studies – Depression Scale; MET=Metabolic equivalent of task; MRI=Magnetic resonance imaging; N=sample size; N[x]=non-REM stage x; PSG=polysomnography; PSQI=Pittsburgh Sleep Quality Index; REM=rapid-eye movement; TST=Total Sleep Time.

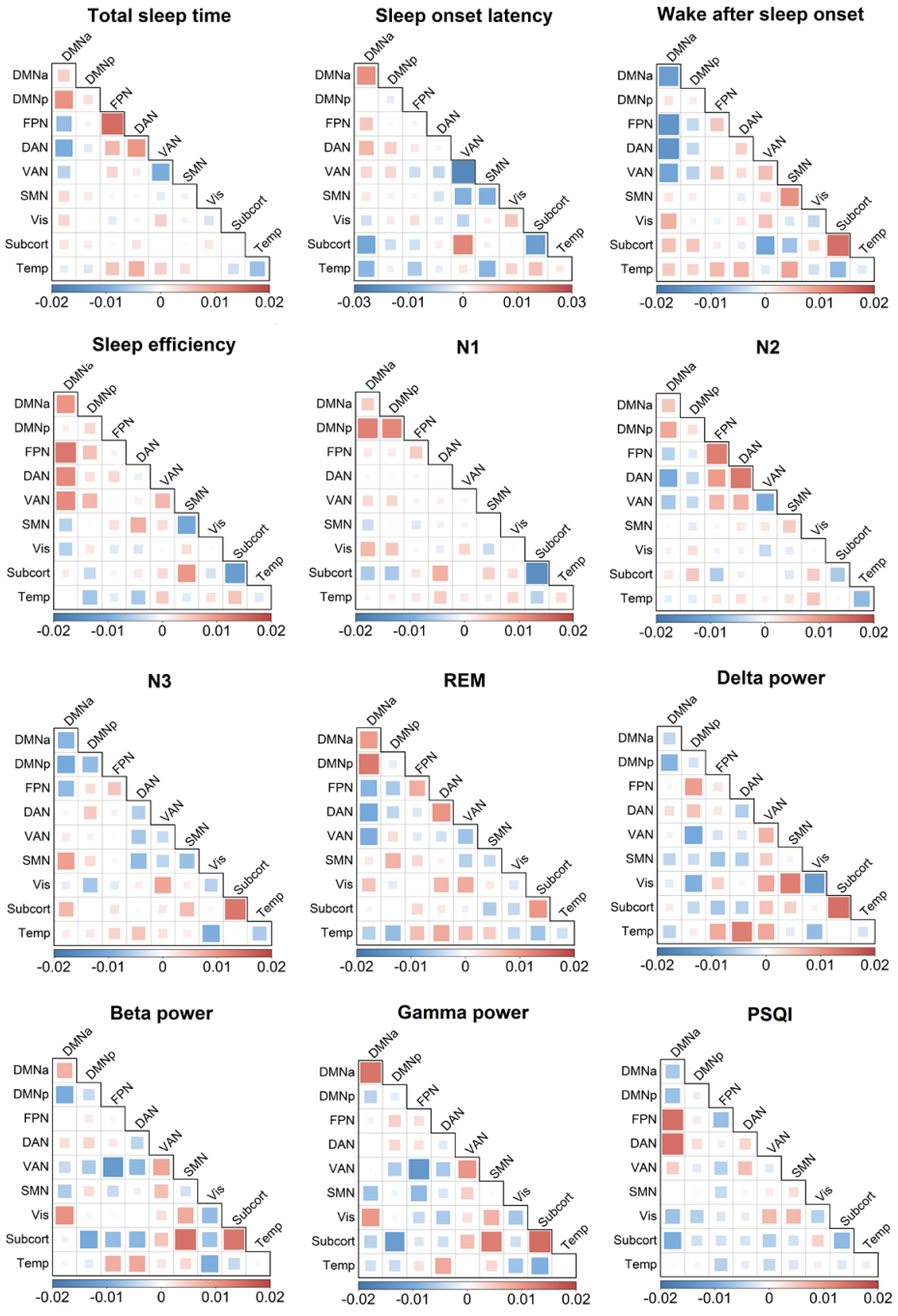

Figure 1. Associations of sleep parameters with functional connectivity between networks

Colors and sizes of blocks correspond to beta coefficients: Red indicates positive, and blue indicates negative associations. Values are obtained using linear regression, adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume. No associations were significant at the level of PFWE-corrected < 0.025. Abbreviations: DMNa=anterior default mode network; DMNp=posterior default mode network; DAN=dorsal attention network; FPN=frontoparietal network; N[x]=non-REM sleep stage x; PSQI=Pittsburgh Sleep Quality Index; REM=rapid eye movement; SMN=sensorimotor network; Subcort=subcortical network; Temp=temporal network, Vis=visual network; VAN=ventral attention network.

Table 2. Associations of sleep parameters with global mean signal amplitude

Sleep measures	Beta (95% CI)	P-value
Objective		
Sleep continuity measures		
Total sleep time	**-0.025 (-0.044; -0.006)**	**5.0e-3**
Sleep onset latency	0.015 (-0.020; 0.049)	0.19
Wake after sleep onset	-0.001 (-0.022; 0.019)	0.45
Sleep efficiency	-0.014 (-0.038; 0.010)	0.14
Sleep stage duration		
N1	-0.013 (-0.034; 0.007)	0.10
N2	-0.013 (-0.032; 0.005)	0.08
N3	-0.009 (-0.030; 0.013)	0.21
REM	-0.015 (-0.034; 0.004)	0.05
Spectral power		
Delta power	0.004 (-0.024; 0.032)	0.39
Beta power	0.013 (-0.013; 0.038)	0.16
Gamma power	0.003 (-0.023; 0.029)	0.41
Subjective		
Sleep complaints (global PSQI score)	0.009 (-0.010; 0.028)	0.18

Values represent difference (95% CI) in mean signal amplitude on a whole-brain level, per standard deviation increase in the determinant. Estimates are obtained using linear regression models adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume.
Bold values indicate statistical significance at P<0.025. Please note that P-values were uncorrected as only the 'global' region was tested. Abbreviations: PSQI=Pittsburgh Sleep Quality Index; Nx=non-REM sleep stage x; REM=Rapid eye-movement.

Table 3. Associations of total sleep time and mean signal amplitude in networks

Total sleep time	Beta (95% CI)	P-value	$P_{FWE\text{-}corrected}$
Networks			
1: Default Mode - anterior	**-0.046 (-0.083; -0.010)**	**5.8e-3**	0.04
2: Default Mode - posterior	-0.017 (-0.039; 0.006)	0.08	0.30
3: Fronto-parietal	-0.013 (-0.040; 0.013)	0.16	0.49
4: Dorsal Attention	-0.014 (-0.041; 0.013)	0.15	0.48
5: Ventral Attention	**-0.051 (-0.077; -0.024)**	**4.0e-4**	**1.2e-3**
6: Sensorimotor	**-0.030 (-0.049; -0.010)**	**1.6e-3**	**8.8e-3**
7: Visual	-0.013 (-0.033; 0.008)	0.12	0.39
8: Subcortical	**-0.021 (-0.036; -0.005)**	**4.2e-3**	**2.5e-2**
9: Temporal	**-0.032 (-0.053; -0.011)**	**1.2e-3**	**8.4e-3**

Values represent difference (95% CI) in mean signal amplitude on a network level, per standard deviation increase in total sleep time. Estimates are obtained using linear regression models and permutation tests, adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume. **Bold** indicates statistical significance at P<0.025.

$P_{FWE\text{-}corrected}$>8.6*e*-3). Additionally adjusting analyses for AHI did not influence global and network-level associations (Supplementary Table 3), and the association within 'node 32' remained highly similar (-0.051 (95% CI -0.075;-0.027); $P_{FWE\text{-}corrected}$=1.2*e*-3).

Posthoc, we explored the contribution of individual sleep stages to the association of total sleep time with mean signal amplitude found in four networks, at both the network and node level. As most of total sleep time was spent in stages REM and N2, these stages contributed most to the association (Fig. 3), yet no association survived multiple testing correction.

Analyzing categorized total sleep time did not suggest non-linearity in the relation with signal amplitude at a global or network level (Supplementary Table 4), supported by testing quadratic terms of total sleep time (global: P=0.27; networks: all $P_{FWE\text{-}corrected}$>0.025).

Actigraphy-estimated longer total sleep time was also associated with lower mean signal amplitude at a global level, driven by similar networks as when derived from PSG (Supplementary Table 5).

Self-reported sleep duration was not associated with mean signal amplitude on a global level (-0.011 (95% CI -0.028; 0.004); P=0.07), nor on a network level (all $P_{FWE\text{-}corrected}$>0.025).

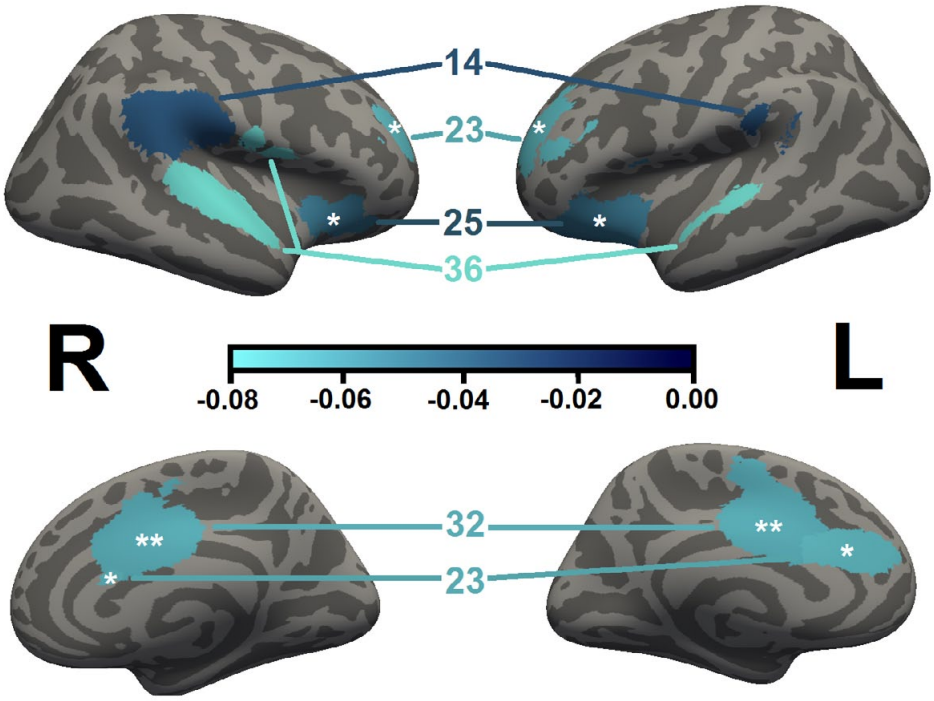

Figure 2. Topographical view of associations of total sleep time with signal amplitude within nodes of the ventral attention network

Negative associations of total sleep time with signal amplitude are shown for all 5 nodes of the ventral attention network on inflated right and left hemispheres, from a lateral (top row) and medial (bottom row) perspective. Lighter colors correspond to larger negative effect sizes (beta coefficients). Asterisks denote statistical significance as: *$P_{FWE\text{-}corrected}$<0.025; **$P_{FWE\text{-}corrected}$<0.00277. Please note that significance levels differ from effect sizes. Values represent difference in signal amplitude in that node per standard deviation increase in total sleep time, and are obtained through linear regression and permutation testing. Coefficients are adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume. Nodes correspond to the following regions (labeled using the probabilistic Harvard-Oxford cortical atlas found at https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases; top three overlapping regions): **Node 14**=Parietal operculum (16%), Posterior (16%) and Anterior (16%) Supramarginal cortex; **Node 36**= Superior Temporal cortex (21%), Temporal Pole (9%), Central Opercular cortex (9%); **Node 25**= Frontal Orbital cortex (28%), Insular cortex (17%), and Frontal Pole (8%); **Node 23**= Frontal pole (29%), Cingulate cortex - anterior division (9%), and Paracingulate cortex (6%); **Node 32**= Cingulate cortex – anterior division (24%), Juxtapositional Lobule (formerly: Supplementary Motor cortex) (13%), and Paracingulate cortex (5%). Threshold of node borders was set at z=5.0.

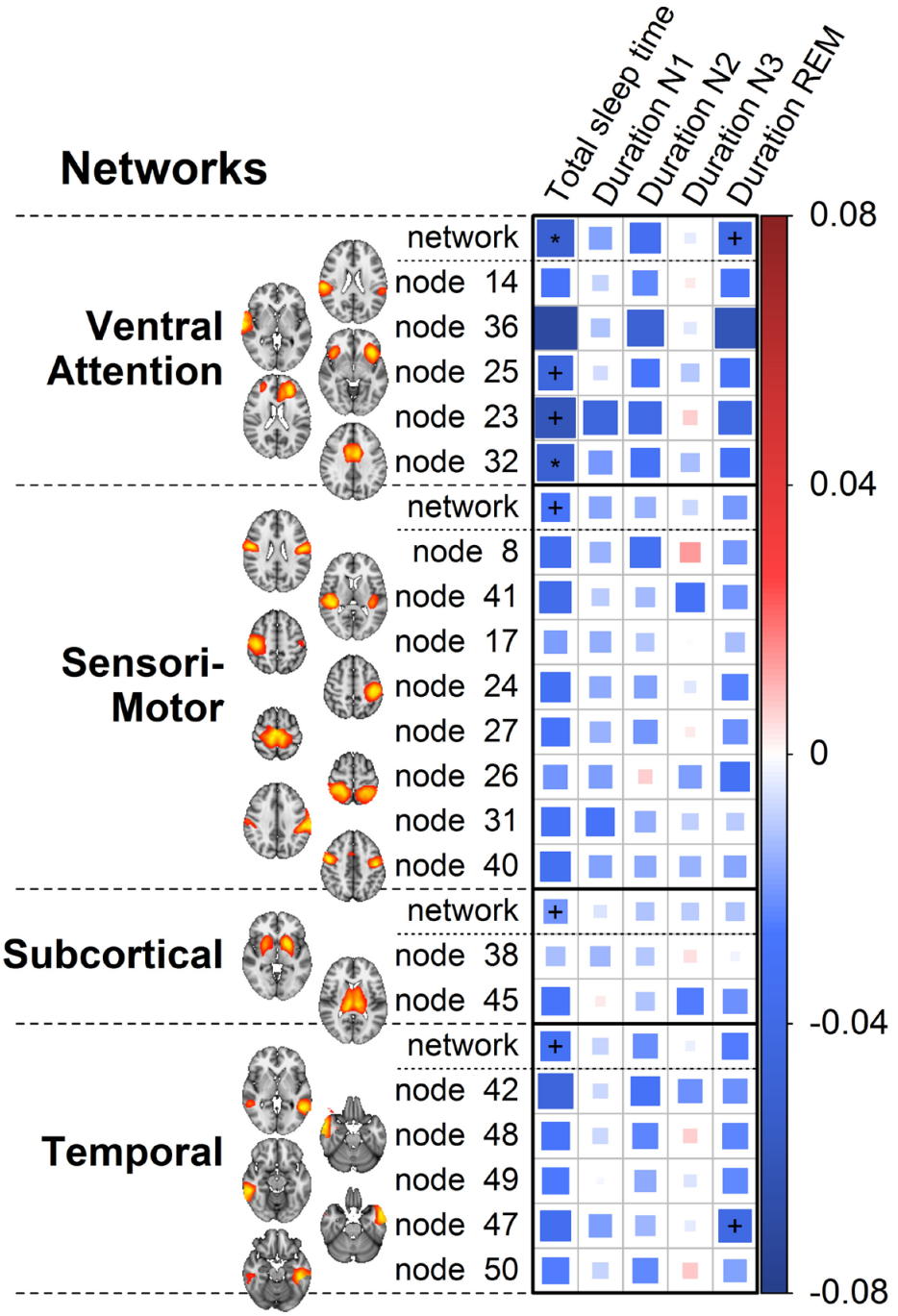

Figure 3. Associations of total sleep time, and duration of sleep stages, with signal amplitude within significant networks, and within their nodes

Associations of total sleep time and sleep stages with (mean) signal amplitude are shown for the four networks with a statistically significant relation. Corresponding nodes are depicted in the axial plane (right = anatomical left) at the level of highest node intensity. Colors and sizes of blocks correspond to effect sizes (beta coefficients): red indicates positive, and blue indicates negative associations.
Values are obtained through linear regression and permutation testing. Coefficients represent difference in signal amplitude in that network or node per standard deviation increase in the sleep parameter, adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume. FWE-corrected P-values for networks were corrected over all 9 networks, and for nodes were corrected for all 50 nodes. Symbols denote: + $P_{FWE\text{-}corrected}<0.025$; *$P_{FWE\text{-}corrected}<0.00277$. Please note that significance levels differ from effect sizes. Abbreviations: Nx= non-REM sleep stage x; REM=rapid eye movement.

DISCUSSION

In this population-based study, we found that PSG-determined longer total sleep time was associated with a lower mean BOLD-signal amplitude during daytime, primarily in the ventral attention network. In contrast, no objective or subjective sleep parameter was associated with functional connectivity between or within networks.

No study previously investigated the relation of objectively measured sleep with intrinsic neural activity measured at median 17 days apart, using a population-based design. In a large-scale study using UK biobank data, self-reported total sleep time was negatively correlated with signal amplitude in networks labeled as sensory/motor, not attentional networks.[12] We found no association for self-reported sleep duration assessed with the PSQI, but to the extent that PSG-derived total sleep time measured a similar construct, differences in study-specific parcellation, attributing the same functional node to different networks, may explain regional differences between studies.

We measured both sleep and rs-fMRI not within a 24-hour timeframe, which makes the association more robust to biases due to variable recording conditions of PSG and rs-fMRI. The association was more pronounced in persons who underwent measurements within a shorter, 1-month time interval, suggesting that effects were short-lived. Yet, both sleep[25,26] and resting state measures[27,28] exhibit 'trait'-like, time-stable properties, supporting that our association may extend beyond a night-to-day effect. Our findings were specific to BOLD-signal amplitude. Momentary increases in BOLD-signal may reflect local, task-triggered neural activity.[29] This amplitude does not refer to momentary increases but to increased *fluctuations over time*. Although its correlates have not been well characterized several observations suggest it is representative of a sleep-deprived state or lower vigilance.[7,30,31] After sleep deprivation, increased lapses in attentional maintenance can be observed[32] and such lapses may be accompanied by repeated intrusions of sleep.[30]

Alternatively, the amount of wakefulness could equally well underlie the association of total sleep time and signal amplitude as it was not driven by a specific sleep stage, and was also found when using actigraphy-estimated habitual total sleep time. Extended wakefulness increases synaptic potentiation,[33] and low-frequency EEG power[34] indicative of more synchronized activity. This power increase is most pronounced medio-frontally as was our association. Also, high amplitude activity on EEG observed in deep sleep indicates more synchronized fluctuations in membrane potential.[35] Against this background, we speculate that the association with BOLD-signal amplitude may also result from more synchronized, infra-slow neural activity during wakefulness.

Although we could not assess temporality in our cross-sectional study, these potential mechanisms favor a temporal association from sleep, or wakefulness, to brain intrinsic neural activity. Yet, the topographical overlap of our findings to the regions involved in the generation and propagation of sleep itself[36,37] may also suggest that signal amplitude determines total sleep time in a population-based, 'non-laboratory' setting. The temporality of the association of objectively estimated total sleep time and regional brain activity, or shared causes, should be studied further.

No sleep parameter was associated with network functional connectivity, in line with previous findings for the PSQI score.[15] Findings differ from experimental sleep deprivation studies that show a consistent impact on subsequent e.g. within-network connectivity of the default mode network.[6] Possibly, sleep deprivation effects may be too short-lived to be detected here. Furthermore, such effects inherently differ from our sleep measures which are more indicative of chronic, stable aspects of sleep. Importantly, methodological heterogeneity in e.g. study design, imaging processing, or modelling approaches may also explain finding null results in contrast to literature, as concluded recently for insomnia neuroimaging findings.[38] Also, bias by lack of adequate control for potential confounders or use of seed-based approaches[38] may have made previous studies more prone to finding false-positive results.

Several methodological considerations deserve mention. First, we did not monitor sleep during rs-fMRI acquisition and cannot rule out contamination of our measures by sleep.[39] Even light sleep stages[40] involve increases in global signal amplitude, consistent over networks. Individuals with a short total sleep time may have been at increased likelihood of falling asleep in the scanner, which may have biased our estimates. However, several observations suggest that contamination less likely explains our findings: i) We found no non-linearity in our associations for total sleep time, indicating that results were not driven by short sleepers only; ii) Total sleep time was not correlated with head motion, which may indicate sleepiness in the scanner[41]; iii) Even light stages of sleep involve substantially altered network connectivity.[39,42] This suggests that, if sleeping in the scanner drove our results for signal amplitude, one might expect to also find associations with functional connectivity between or within networks. Yet, we found none,

indicating that likely few participants slept during rs-fMRI acquisition. We ensured, by addressing participants, that they were awake at the start of rs-fMRI acquisition. Further vigilance monitoring with concomitant EEG was not deemed necessary nor feasible due to the population-based nature of our study. Second, we could not assess the influence of sleep the night preceding rs-fMRI acquisition. Third, performing fMRI at 1.5T instead of higher field strengths, and not controlling for variable conditions during rs-fMRI acquisition, may have reduced our sensitivity to detect associations. Similarly, retrospective assessment of sleep with the PSQI over the previous 4 weeks may have reduced chances to detect cross-sectional associations for PSQI-derived measures. Third, we could not assess how local differences in gray matter influenced our estimates beyond global volume. Study strengths include using PSG to study sleep over a broad and 'real-life' spectrum in a population-based study population, having substantial statistical power to detect small effect sizes, and adjusting for multiple potential confounders.

We conclude that, in the general middle-aged and elderly population, total sleep time affects the repertoire of (pre)frontal brain activity, or vice versa, beyond a night-to-day effect. At the same time, our results suggest there is no clear association of objective and subjective measures of sleep with functional connectivity between or within resting-state networks.

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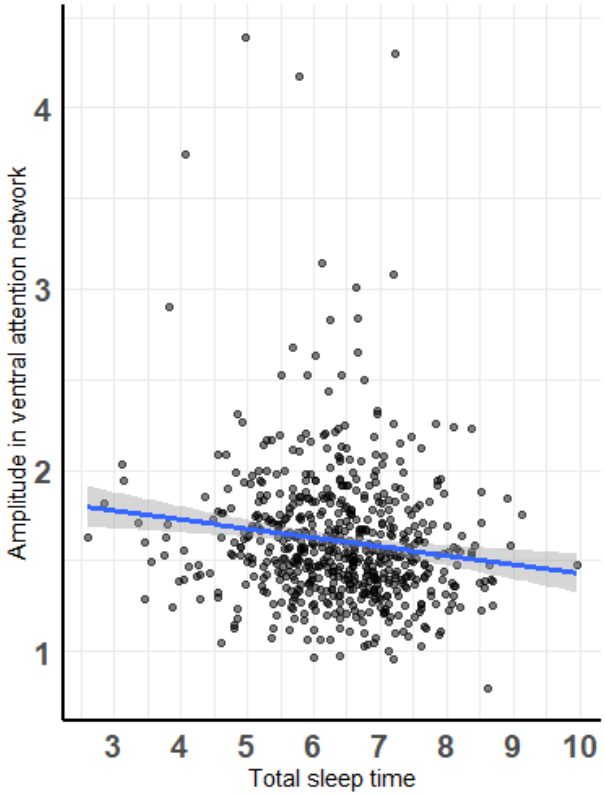
SUPPLEMENTARY MATERIAL

Supplementary Table 1. Correlations amongst sleep parameters and MRI quality parameters

TST	*TST*													
SOL	**-0.20**	*SOL*												
WASO	**-0.30**	**0.14**	*WASO*											
SE	**0.52**	**-0.48**	**-0.85**	*SE*										
N1	0.08	-0.03	**0.33**	**-0.20**	*N1*									
N2	**0.69**	-0.17	**-0.26**	**0.41**	0.02	*N2*								
N3	**0.18**	-0.01	**-0.19**	**0.19**	**-0.42**	**-0.33**	*N3*							
REM	**0.63**	**-0.14**	**-0.29**	**0.42**	-0.08	**0.30**	0.06	*REM*						
Delta	**0.15**	0.01	-0.10	0.11	**-0.44**	-0.07	**0.58**	0.03	*Delta*					
Beta	-0.04	0.09	**0.15**	**-0.13**	**-0.17**	**-0.14**	**0.26**	-0.04	**0.53**	*Beta*				
Gam	-0.06	0.11	**0.17**	**-0.15**	-0.11	**-0.17**	**0.22**	-0.06	**0.50**	**0.84**	*Gam*			
PSQI	-0.06	0.06	0.08	-0.11	-0.09	-0.02	0.06	-0.09	0.12	**0.17**	0.14	*PSQI*		
Gh	0.01	-0.06	0.02	0.03	0.11	0.07	**-0.17**	0.00	-0.12	-0.13	**-0.16**	**-0.14**	*Gh*	
FD	0.02	-0.03	0.00	0.01	0.08	0.01	-0.01	-0.02	-0.05	-0.12	-0.10	0.06	-0.04	*FD*

Spearman correlation values for polysomnography-derived parameters (sleep continuity measures, absolute sleep stage duration, spectral powers in three frequency bands), global PSQI score, and two MRI quality parameters. Values are obtained for complete case pairwise comparisons, using original variable distributions. Comparisons between spectral power frequency bands (n=560) and global PSQI score (n=603) were calculated in=543. **Bold** indicates statistical significance at P<0.001.

Abbreviations: Delta/Beta/Gam=Absolute spectral power in the delta/beta/gamma frequency band; FD=Frame-wise head displacement; Gh=ghost-to-signal ratio; Nx=non-REM sleep stage x sleep duration; PSQI=Global Pittsburgh Sleep Quality Index score; REM=Rapid eye movement sleep duration; SE=Sleep efficiency; SOL=Sleep onset latency; TST=Total sleep time; WASO=Wake after sleep onset



Supplementary Figure 1. Scatterplot of association of total sleep time with mean signal amplitude in the ventral attention network

The data points graphically depict the relation of longer total sleep time with mean signal amplitude in the ventral attention network. The regression line and corresponding shaded 95% confidence interval (CI) show the average relation obtained after linear regression. The multivariate adjusted linear association was statistically significant association after correcting for multiple testing only in this network (per standard deviation increase of total sleep time: -0.051 mean difference in signal amplitude (95% CI -0.077l -0.024); $P_{FWE\text{-}corrected}$=1.2e-3. Total sleep time is depicted in hours.

Supplementary Table 2. Associations of sleep parameters with mean signal amplitude at the global and network level, restricted to participants with a shorter time interval between sleep and rs-fMRI measurement

Level	Sleep parameters	Beta (95% CI)	P	$P_{FWE\text{-}corrected}$
Global	Total sleep time	**-0.033 (-0.055; -0.011)**	**2.4e-3**	-
	Sleep onset latency	-0.009 (-0.050; 0.032)	0.35	-
	Wake after sleep onset	-0.001 (-0.025; 0.023)	0.47	-
	Sleep efficiency	-0.005 (-0.033; 0.024)	0.38	-
	Stage N1 duration	-0.010 (-0.034; 0.014)	0.19	-
	Stage N2 duration	**-0.024 (-0.046; -0.003)**	**1.2e-2**	-
	Stage N3 duration	-0.007 (-0.031; 0.018)	0.30	-
	Stage REM duration	-0.016 (-0.038; 0.006)	0.08	-
	Spectral delta power	-0.003 (-0.031; 0.026)	0.44	-
	Spectral beta power	0.011 (-0.020; 0.041)	0.24	-
	Spectral gamma power	-0.009 (-0.041; 0.023)	0.31	-
	Global PSQI score	0.014 (-0.009; 0.037)	0.12	-
Network	Total sleep time			
	1: Default mode – anterior	**-0.058 (-0.100; -0.015)**	**5.2e-3**	**2.9e-2**
	2: Default mode – posterior	-0.025 (-0.051; 0.002)	0.04	0.17
	3: Frontoparietal	-0.024 (-0.054; 0.006)	0.06	0.25
	4: Dorsal attention	-0.023 (-0.055; 0.009)	0.07	0.29
	5: Ventral attention	**-0.062 (-0.094; -0.030)**	**6.0e-4**	**1.6e-3**
	6: Sensorimotor	**-0.039 (-0.061; -0.017)**	**4.0e-4**	**2.4e-3**
	7: Visual	-0.011 (-0.034; 0.012)	0.17	0.51
	8: Subcortical	**-0.029 (-0.047; -0.012)**	**6.0e-4**	**4.8e-3**
	9: Temporal	**-0.045 (-0.070; -0.020)**	**8.0e-4**	**2.2e-3**
	Stage N2 duration			
	1: Default Mode – anterior	-0.039 (-0.082; 0.003)	0.03	0.16
	2: Default Mode – posterior	-0.023 (-0.049; 0.004)	0.05	0.20
	3: Fronto-parietal	-0.025 (-0.055; 0.004)	0.04	0.20
	4: Dorsal Attention	-0.008 (-0.039; 0.023)	0.30	0.71
	5: Ventral Attention	**-0.049 (-0.081; -0.017)**	**1.2e-3**	**9.4e-3**
	6: Sensorimotor	**-0.025 (-0.047; -0.003)**	**1.3e-2**	0.07
	7: Visual	-0.003 (-0.026; 0.019)	0.39	0.79
	8: Subcortical	**-0.023 (-0.041; -0.006)**	**4.0e-3**	**2.6e-2**
	9: Temporal	**-0.038 (-0.063; -0.013)**	**1.6e-3**	**9.8e-3**

We analyzed associations at the global level, and further explored significant sleep parameters (total sleep time and stage N2 duration) at the network level. The absolute time interval between sleep and rs-fMRI measurement was ≤1 month for polysomnography (n=450 [72%], n=406 for spectral power variables [65%]), and ≤6 months for PSQI (n= 430 [69%]). Values represent difference (95% CI) in mean signal amplitude per standard deviation increase in the sleep parameter. Estimates are obtained using linear regression models and permutation tests, adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume. **Bold** indicates statistical significance at P<0.025. Please note that P-values at the global level were uncorrected as only the 'global' region was tested. Abbreviations: N=sample size; Nx=non-REM sleep stage x; PSQI=Pittsburgh Sleep Quality Index; REM=rapid eye movement

Supplementary Table 3. Associations of sleep parameters with mean signal amplitude at the global and network level, additionally adjusted for apnea-hypopnea index

Level	Sleep parameter	Beta (95% CI)	P	$P_{FWE\text{-}corrected}$
Global	Total sleep time	**-0.025 (-0.044; -0.006)**	**5.2e-3**	-
	Sleep onset latency	0.014 (-0.020; 0.049)	0.20	-
	Wake after sleep onset	-0.001 (-0.022; 0.019)	0.45	-
	Sleep efficiency	-0.014 (-0.038; 0.011)	0.14	-
	Stage N1 duration	-0.013 (-0.034; 0.007)	0.10	-
	Stage N2 duration	-0.013 (-0.032; 0.005)	0.08	-
	Stage N3 duration	-0.009 (-0.030; 0.012)	0.21	-
	Stage REM duration	-0.015 (-0.034; 0.004)	0.06	-
	Spectral delta power	0.004 (-0.024; 0.031)	0.40	-
	Spectral beta power	0.014 (-0.011; 0.039)	0.14	-
	Spectral gamma power	0.006 (-0.020; 0.032)	0.33	-
	Global PSQI score	0.009 (-0.010; 0.028)	0.18	-
Network	Total sleep time			
	1: Default mode – anterior	**-0.047 (-0.083; -0.01)**	**5.6e-3**	0.04
	2: Default mode – posterior	-0.016 (-0.039; 0.007)	0.09	0.32
	3: Frontoparietal	-0.013 (-0.040; 0.013)	0.16	0.50
	4: Dorsal attention	-0.014 (-0.042; 0.013)	0.15	0.47
	5: Ventral attention	**-0.051 (-0.078; -0.025)**	**4.0e-4**	**1.0e-3**
	6: Sensorimotor	**-0.030 (-0.049; -0.010)**	**1.6e-3**	**8.8e-3**
	7: Visual	-0.012 (-0.033; 0.008)	0.12	0.40
	8: Subcortical	**-0.020 (-0.036; -0.005)**	**5.0e-3**	0.03
	9: Temporal	**-0.032 (-0.052; -0.011)**	**1.6e-3**	**9.2e-3**

We analyzed associations at the global level, and further explored significant sleep parameters (total sleep time) at the network level. Values represent difference (95% CI) in mean signal amplitude per standard deviation increase in the sleep parameter. Estimates are obtained using linear regression models and permutation tests, adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume. **Bold** indicates statistical significance at P<0.025. Please note that P-values at the global level were uncorrected as only the 'global' region was tested.

Abbreviations: N=sample size; Nx=non-REM sleep stage x; PSQI=Pittsburgh Sleep Quality Index; REM=rapid eye movement

Supplementary Table 4. Effect sizes of associations of categorized total sleep time with mean signal amplitude, at both the global and network level

Level	Categories (quintiles) of total sleep time in hours				
	<5.6	5.6-6.2	6.2-6.6	6.6-7.2	>7.2
Global	0.035	0.006	0.000 (ref)	-0.046	-0.035
Network					
1: Default mode - anterior	0.020	-0.041	0.000 (ref)	-0.096	-0.116
2: Default mode - posterior	0.047	0.042	0.000 (ref)	-0.014	0.014
3: Frontoparietal	0.031	0.006	0.000 (ref)	-0.029	-0.017
4: Dorsal attention	0.002	-0.047	0.000 (ref)	-0.074	-0.060
5: Ventral attention	0.066	0.031	0.000 (ref)	-0.067	-0.055
6: Sensorimotor	0.052	0.016	0.000 (ref)	-0.031	-0.031
7: Visual	0.004	0.000	0.000 (ref)	-0.048	-0.035
8: Subcortical	0.031	-0.001	0.000 (ref)	-0.041	-0.025
9: Temporal	0.055	0.017	0.000 (ref)	-0.043	-0.025

Associations of categories (quintiles) of total sleep time with mean signal amplitude at the global level, and the network level. Values represent difference in mean signal amplitude for that quintile of total sleep time, referenced to the middle quintile. Values are obtained through linear regression adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume.

Supplementary Table 5. Associations of actigraphy-estimated sleep parameters with mean signal amplitude at the global and network level, in n=428 persons

Level	Sleep parameters	Beta (95% CI)	P	$P_{FWE\text{-}corrected}$
Global	Total sleep time	**-0.025 (-0.048; -0.001)**	**2.3e-2**	-
Network	Total sleep time			
	1: DMN – anterior	**-0.068 (-0.113; -0.023)**	**1.0e-3**	**8.6e-3**
	2: DMN – posterior	0.000 (-0.029; 0.029)	0.50	0.87
	3: Frontoparietal	-0.006 (-0.038; 0.027)	0.35	0.75
	4: Dorsal attention	-0.034 (-0.069; 0.000)	0.03	0.12
	5: Ventral attention	**-0.048 (-0.080; -0.016)**	**1.0e-3**	**9.4e-3**
	6: Sensorimotor	**-0.031 (-0.055; -0.007)**	**5.2e-3**	**3.6e-2**
	7: Visual	-0.005 (-0.032; 0.021)	0.33	0.73
	8: Subcortical	**-0.032 (-0.050; -0.014)**	**4.0e-4**	**2.2e-3**
	9: Temporal	-0.024 (-0.051; 0.003)	0.04	0.18

We analyzed associations at the global level, and further explored total sleep time at the network level. Values represent difference (95% CI) in mean signal amplitude per standard deviation increase in the sleep parameter. Estimates are obtained using linear regression models and permutation tests, adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume.
Bold indicates statistical significance at P<0.025. Please note that P-values at the global level were uncorrected as only the 'global' region was tested. Abbreviations: DMN=Default mode network; N=sample size; Nx=non-REM sleep stage x; PSQI=Pittsburgh Sleep Quality Index; REM=rapid eye movement

Supplementary Methods

Measurement of potential confounders and effect-modifiers

Potential confounders were selected based on impacting sleep derived from PSG, the neurovascular process underlying the fMRI BOLD-signal, or both.[1,2] Covariates, unless mentioned otherwise, were measured at the home interview or center visit, mostly before PSG. Age was determined at the polysomnography measurement. Values for ghost-to-signal ratio and mean frame-wise head displacement were obtained during fMRI preprocessing. Educational attainment was self-reported in four levels, expressed in corresponding average years (7, 9, 13, or 19). Habitual alcohol consumption was quantified with the Food Frequency Questionnaire[3] as grams/day intake. Physical activity was queried[4] and quantified in standardized measures of activity intensity (metabolic activity of task per week).[5] Systolic blood pressure in mm Hg was the average of two right-arm measurements when sitting up. Body mass index was calculated from measured weight and height (kg/m^2). Diabetes mellitus was defined as a fasting serum glucose level ≥7.0 mmol/L and/or self-reported use of anti-diabetic medication. Intracranial and supratentorial gray matter volume were obtained from structural MRI

(T1-weighted sequence) segmentations.[6] Depressive symptoms were assessed with the validated Dutch version[7] of the Centre for Epidemiological Studies Depression Scale.[8] Self-reported use of any antidepressant or hypnotic medication during the night of PSG was queried in an accessory sleep diary.

Statistical analysis

First, we calculated pair-wise correlations between all sleep determinants, and included frame-wise head displacement and ghost-to-signal ratio to examine how sleep related to MRI-parameters.[9] Main analyses were performed using general linear models with the intrinsic neural activity parameter as a dependent variable. We used group-level non-parametric permutation testing (n=5,000) implemented by FSL's randomise with family-wise error (FWE) corrected P-values to evaluate significance when testing associations in multiple regions within one topographical scale (i.e. at the network- or node-level). We chose sleep determinants informed by prior research.[10,11] Spectral power in the delta, beta and gamma bands were chosen based on the role of slow-wave activity on synaptic potentiation[12,13] and the role of high frequency bands as potential electrophysiological markers of hyperarousal in insomnia.[14-16] Lastly, we investigated global PSQI score as a measure of subjective sleep quality.

Thresholds for statistical significance and further exploring regional effects were a compromise between missing regional effects that may be 'averaged out' on a larger scale and type I error: Associations were regionally explored with $P_{FWE\text{-}corrected}<0.025$, halving the *alpha* of 0.05, as tests were performed one-tailed. A second, more stringent, significance threshold was defined to account for testing multiple sleep aspects in this study. For this, we computed the number of effective tests[17] (M_{eff}=9.23) based on Pearson correlations between the 12 sleep determinants, subsequently applied a Sidak correction,[18] and halved the new alpha level for two-tailed tests (P<0.00277).

In sensitivity analyses, we tested the robustness of findings by repeating the main analyses after including only participants with PSG and rs-fMRI measurements <1 month apart (n=450 for sleep stage scoring, n=425 for spectral analysis), or <6 months apart for PSQI (n=438). We additionally adjusted analyses for depressive symptoms and self-reported use of any versus no antidepressant or hypnotic medication at the night of PSG considering these factors may relate to both sleep and rs-fMRI parameters.[19,20] We also adjusted analyses for the apnea-hypopnea index, a prevalent indicator of potential obstructive sleep apnea in our study population.[21]

Posthoc, exploratory analyses included modeling a quadratic term of total sleep time (TST*TST), which was added besides the main effects term of TST, to statistically test potential non-linearity.

To minimize the effect of outliers, we winsorized outliers (changed to approach the mean) to 3 standard deviations from the mean. Sleep parameters were then standard-

ized (subtracting the sample mean and dividing by the standard deviation) to facilitate comparison of effect sizes. Missing data on covariates (mean=3%) were imputed using 5 multiple imputations, based on all analysis variables. Missing values imputation was performed with IBM SPSS Statistics version 22.0 (IBM Corp, Armonk, NY). Brain topographical depictions were created using Freesurfer Freeview. Figures including heat maps were created using R.

Supplementary references

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