Intrinsic brain connectivity after partial sleep deprivation in young and older adults: results from the Stockholm Sleepy Brain study

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Abstract

Background: Sleep deprivation has been reported to affect intrinsic brain connectivity, notably in the default mode network, but studies to date have shown inconsistent effects and have largely included young participants.

Aims: We aimed to investigate effects of partial sleep deprivation on intrinsic brain connectivity in young and older participants.

Methods: Participants aged 20-30 (*n* = 30) and 65-75 (*n* = 23) years underwent partial sleep deprivation (3 h sleep) in a cross-over design, with two resting state functional magnetic resonance imaging (fMRI) runs in each session. We assessed intrinsic brain connectivity using independent components analysis (ICA) as well as seed-region analyses of functional connectivity, and also analysed global signal variability, regional homogeneity, and the amplitude of low-frequency fluctuations.

Results: Sleep deprivation caused increased global signal variability. In contrast to previous smaller studies, sleep deprivation did not cause major changes in investigated resting state networks, nor did it cause changes in regional homogeneity. Younger participants had higher functional connectivity in most examined resting state networks, as well as higher regional homogeneity in brain areas including anterior and posterior cingulate cortex.

Conclusion: We show for the first time that partial sleep deprivation caused increased global signal variability. This outcome should be examined as a potential biomarker for sleepiness using independent data.

Key words: Sleep, Sleepiness, Resting state, Functional connectivity, Aging

1 Introduction

1.1 Background

Sleep problems are a public health issue affecting about one third of the general population, of which about one in three reports serious sleep problems (Léger and Bayon, 2010, SBU, 2010). Impaired or shortened sleep is a risk factor for mortality and for a number of diseases (Buysse et al., 2010), as well as accidents (Åkerstedt et al., 2011). These risks appear to be mediated by impaired biological restoration/recovery (Buysse et al., 2010). Effects of sleep loss include an increasing number of short lapses of attention (microsleeps) (Lim and Dinges, 2008), as well as increased levels of EEG alpha and theta activity; slow, rolling eye movements; and increased subjective sleepiness (Åkerstedt and Gillberg, 1990).

There is no well-defined brain area associated with sleepiness, but early studies of total sleep deprivation have shown reduced glucose uptake in large areas of the brain, including prefrontal and parietal areas (Thomas et al., 1993). Intrinsic connectivity refers to functional connectivity in the resting state, i.e. when participants are not presented with any changing stimuli. Intrinsic connectivity after sleep deprivation has been investigated in several earlier studies, which have focused on different connectivity measures and brain areas. The most consistent finding appears to be that total sleep deprivation caused reduced connectivity within the default mode network (Sämann et al., 2010, De Havas et al., 2012, Yeo et al., 2015). Other findings include increased regional homogeneity (ReHo) in different brain areas following sleep deprivation (Dai et al., 2012, Gao et al., 2015), and changes in connectivity between the thalamus (Shao et al., 2013) and amygdala (Shao et al., 2014, Lei et al., 2015) and cortical areas.

It is an interesting question whether reduced connectivity, observed in previous studies, is associated with subjective sleepiness, which is a sensitive indicator of sleep loss (Åkerstedt et al 2014). There is also a question whether participants in previous studies were able to consistently maintain wakefulness during resting state scanning, not least in light of findings by Tagliazucchi and Laufs (2014), showing that about 50% of non-sleep-deprived participants fall asleep within 10 minutes of resting state imaging. Prior studies of intrinsic connectivity after sleep deprivation have mainly relied on self-report to rule out episodes of falling asleep during scanning. Previous studies on intrinsic brain connectivity after sleep loss have focused on total sleep deprivation, while partial sleep loss is much more common in everyday life. An additional observation is that the role of age has not been investigated in studies of sleep deprivation and connectivity. It appears that sleepiness is reduced in older individuals both when sleeping normally and after sleep deprivation (Dijk et al 2010). There is as yet no data to inform theory on this question, but contrary to intuition, younger individuals seem more susceptible to sleep loss in terms of physiological and self-reported sleepiness (Dijk et al., 2010). Furthermore, sleep duration decreases with age (Ohayon et al., 2004) and the ability to produce sleep under optimal conditions also falls with age (Klerman and Dijk, 2008), suggesting that sleep need is reduced.

The fMRI global signal during rest has been the topic of controversy as a nuisance regressor. Murphy et al. (2009) reported that global signal regression may introduce artifactual

anti-correlations between resting state networks (see also Weissenbacher et al., 2009). Saad et al. 2012 reported that global signal regression may also introduce bias in comparisons between groups and time-points, and Guo et al. (2012) reported that global signal regression reduced re-test reliability of salience network estimation in 29 older adults scanned with an interval of a year. In this context, it deserves to be mentioned that the results from the studies that first showed negative correlations between resting state networks, primarily between the default mode and task-positive networks (Fransson, 2005, Fox et al., 2005) have subsequently been reproduced without use of global brain mean signal regression (e.g. Fox et al., 2009, Chai et al., 2012). However, recently, the global signal has become a focus for interest in itself. Schölvink et al. (2010) reported a correlation between spatially widespread BOLD signal changes and spontaneous fluctuations in local field potentials in monkeys, mainly in the high gamma frequency band (Schölvink et al., 2010). Furthermore, Wong et al. reported 2012 that global signal variability was decreased by caffeine and furthermore 2013 and 2016 that global signal variability was negatively correlated to EEG measures of vigilance. In addition, Fukunaga et al. (2006) found an increased BOLD signal amplitude in those individuals that were asleep at the end of a resting state session. These findings suggest that global signal variability is higher in sleepiness, but the effect of sleep deprivation on global signal variability has not been investigated previously, nor has its relation to subjective sleepiness.

The role of prior sleep, apart from sleep deprivation, may also be of interest in relation to resting state connectivity. Thus, Killgore et al. (2012) showed that self-reported shorter sleep duration prior to imaging was associated to reduced connectivity. No studies have, however, studied the contents of prior sleep with polysomnography, the gold standard for sleep measurement. Both sleep fragmentation (Bonnet and Arand, 2003) and suppression of N3 ("deep") sleep (Dijk et al., 2010) increase physiological sleepiness. Partial sleep deprivation (PSD) allowing 2 hours of sleep strongly increase physiological and self-reported sleepiness, while PSD allowing 4 hours of sleep only gives marginal increases (Härmä et al., 1998). Thus, it is an interesting question whether sleep reduction to < 4h causes reduced resting state connectivity.

This study investigated intrinsic brain connectivity measures and global signal variability after partial sleep deprivation and in relation to PSG and reported sleepiness in the Stockholm Sleepy Brain Study 1, a brain imaging study including younger (20-30 years) and older (65-75 years) healthy volunteers.

1.2 Aims

We aimed to investigate the effects of partial sleep deprivation on intrinsic brain connectivity. Specifically, we hypothesised that sleep deprivation would cause

- decreased connectivity within the default mode (DMN), salience, frontoparietal attention, and executive control networks.
- decreased anticorrelation between DMN and the task-positive network (TPN) during resting state.
- changes in thalamocortical connectivity.

- changes in connectivity from amygdala, specifically decreased connectivity between amygdala and prefrontal cortex.
- changes in regional homogeneity
- increased global signal variability

Furthermore, we hypothesised that the above-mentioned measures would correlate to PSG measures of sleep and to self-rated sleepiness, and that older participants would have lower functional connectivity and be less sensitive to sleep deprivation. We also exploratively investigated the amplitude of low-frequency fluctuations (ALFF).

2 Materials and methods

2.1 Study design

The study was a cross-over comparison between 3 h partial sleep deprivation and full sleep. This particular sleep duration was chosen because it appears that clear effects on physiological and subjective sleepiness require < 4 h of total sleep time (TST) (Härme et al 1998), because it is highly relevant for sleep problems in the population, and because pilot testing suggested partial sleep deprivation would be less likely to cause participants to fall asleep during the experiment compared to total sleep deprivation. Participants were randomised to undergo both conditions in a counterbalanced order, with an interval of approximately one month. In the interest of ecological validity, participants slept in their own homes in both conditions. Sleep was monitored using ambulatory polysomnography. In the sleep deprivation condition, participants were instructed to go to bed 3 h before the time they would usually get up, and then to get up at that time. MRI imaging was performed in the evening following sleep deprivation or normal sleep (approximately between 18:00 and 21:00), in order to avoid confounding by circadian rhythms (Blautzik et al., 2013, Hodkinson et al., 2014). Experimenters at the MRI scanner were blinded to participants' sleep condition.

The project was preregistered at clinicaltrials.gov (https://clinicaltrials.gov/ct2/show/NCT02000076), with a full list of hypotheses and an analysis plan available on the open science framework (https://osf.io/zuf7t/). Hypotheses were updated after data collection but before data analysis. The study was approved by the Regional Ethics Review board of Stockholm (2012/1098-31/2). Methods, data, and technical validation have been reported in detail in a previous manuscript (Nilsonne et al. 2016).

2.2 Participants

As described in Nilsonne et al. (2016), participants were recruited by poster advertising on campus sites in Stockholm, on the studentkaninen.se website, and in newspaper ads. Prospective participants were screened for inclusion/exclusion criteria using an online form and eligibility was confirmed in an interview upon arrival to the scanning site. Criteria for inclusion were, first, those required to undergo fMRI procedures and to use the hand-held response box, namely: no ferromagnetic items in body, not claustrophobic, not pregnant, no refractive error that could not be corrected using contact lenses, not color-blind, and right-handed. In addition, participants were required to be 20-30 or 65-75 years old (inclusive), to have no current or past psychiatric or neurological illness, including addiction, to not have hypertension or diabetes, to

not use psychoactive or immune-modulatory drugs, to not use nicotine every day, and to have a lower habitual daily caffeine intake corresponding to 4 cups of coffee at most. A further criterion was to not study, have studied, or be occupied in the fields of psychology, behavioural science, or medicine, including nursing and other allied fields. The reason for this was that participants with a background in psychology might try to "see through" the experimental paradigm, whereas participants with a background in medicine may have a less strong emotional response to pictures showing needles or injuries, which were used in two of the experiments not reported in the present paper. The insomnia severity index (ISI) (Bastien et al., 2001, Trott, 2009), the depression subscale of the Hospital Anxiety and Depression scale (HADS) (Zigmond and Snaith, 1983, Lisspers et al., 1997) and the Karolinska Sleep Questionnaire (KSQ) (Åkerstedt et al., 1998) were used to exclude participants with insomnia symptoms, out-of-circadian sleep patterns, or excessive snoring (see 2.3). For practical reasons, participants were also required to understand and speak Swedish fluently and to live in the greater Stockholm area. Participants were paid 2500 SEK (approx. 280 Euro/360 USD), subject to tax. They were also offered taxi travel to and from the MRI imaging center, in order to avoid traffic incidents following sleep deprivation.

2.3 Sleep measures

As described in Åkerstedt et al. (manuscript in preparation), polysomnography (PSG) recording took place in the homes of the participants using a solid state, portable sleep recorder (Embla). Standard electrode (silver/silver chloride) montage for EEG sleep recording was used (C3, C4 referenced to the contralateral mastoid). In addition, two sub-mental electrodes were used for electromyography (EMG) and one electrode at each of the outer canthi of the eyes were used for electrooculography (EOG). Sleep staging, respiratory and arousal analysis were performed according to the classification criteria of the American Academy of Sleep Medicine (AASM) using the computer-assisted sleep classification system Somnolyzer 24 × 7 (Anderer et al., 2005, 2010). To adapt to AASM scoring, F4 was interpolated. Here the terminology N1, N2, and N3 is used for sleep stages 1-3. Wake within the total sleep period (WTSP) represents time awake between sleep onset and offset and this value is expressed in percent of the total sleep period (TSP). Shifts from any of the sleep stages to wake were expressed as awakenings per hour.

2.4 Experimental task

Two resting state sessions were performed on each of the two visits to the MRI scanner. The first session was in the beginning of scanning, preceded by a 4 min anatomical scan which allowed the participants to acclimatize to the scanner environment. The second session was at the end of scanning, following approx. 1 hour of experiments using emotional stimuli, which will be reported elsewhere. Participants were instructed to look at a fixation cross presented against a gray background, presented using goggles (NordicNeuroLab). During scanning, participants were monitored by eye-tracking. In case of eye-closures of more than approx. 5 seconds, the MRI operator spoke a wake-up call through the participant's headphones. This happened once during resting state scanning among the included participants. In the first run, the resting state acquisition period lasted for 8 minutes with no interruptions. In the second run, also of 8

minutes, participants were asked to rate their sleepiness every 2 minutes with the Karolinska Sleepiness Scale (KSS) (Åkerstedt and Gillberg, 1990, Åkerstedt et al., 2014). This is a single-item question with 9 ordinal anchored response alternatives.

2.5 MRI acquisition

We used a General Electric Discovery 3 T MRI scanner. Echo-planar images were acquired using the following settings: flip angle 75, TE 30, TR 2.5 s, field of view 28.8 cm, slice thickness 3 mm, 49 slices, interleaved bottom → up. T1-weighted anatomical scans were acquired with a sagittal BRAVO sequence, field of view 24 cm, slice thickness 1 mm, interleaved bottom → up.

2.6 Preprocessing

To remove task-related interference, volumes scanned during KSS ratings in the second run were cut out of the time series, including two volumes before and four after each rating event. This procedure removed spikes in the time-series for visual and motor networks, occurring at the time of ratings (not shown). We note that a consecutive time-series is not an assumption underlying such analyses of connectivity as were performed on these data. Remaining volumes were concatenated and in order to balance data, each series was trimmed down to the lowest number of remaining scans in any one session, which was 163, corresponding to 7 minutes and 20 seconds. Data were preprocessed in SPM12 using the DPARSF toolbox (Chao-Gan and Yu-Feng, 2010). Functional images were slice time corrected, realigned, normalized using DARTEL (Ashburner, 2007), resampled to 3x3x3 mm voxel size, spatially smoothed with a 6x6x6 mm kernel, detrended, frequency filtered (0.01-0.1 Hz), and regressed on nuisance covariates including six motion regressors and gray and white matter signal using DARTEL-obtained segmentation. Participants with more than 40 volumes (25%) with framewise displacement (FD) ≥ 0.5 mm were excluded from analysis (n = 15 out of 68, leaving n = 53 for analysis). Remaining volumes with FD ≥ 0.5 mm were cut after nuisance regression and interpolated using cubic splines. To further reduce the risk of motion confounding, FD parameters were carried forward as regressors of no interest in 2nd level analyses.

2.7 Independent component analysis (ICA)

ICA was performed using the GIFT toolbox (Calhoun et al., 2001). 20 independent components were estimated using the Infomax algorithm and the ICASSO approach, with a gray substance mask derived from DARTEL segmentation (see 2.6 above). Following back-reconstruction using spatio-temporal regression, components of interest were extracted for each subject. 2nd level analyses were performed in SPM12. In 2nd level analyses, masks for specific networks were used based on a previously published parcellation (Shirer et al., 2012), illustrated in supplementary figure 1. We also investigated effects within the whole gray substance mask.

2.9 Seed-based analyses

Seed regions for the default mode network (DMN) and the anticorrelated task-positive network (TPN) were based on a previous report on the effect of sleep deprivation on functional connectivity (De Havas et al., 2012). Seed regions were 3x3x3 mm cubic centered on coordinates reported by De Havas et al., 2012, see supplemental table 1. In addition, following

Shao et al., 2013, we defined a thalamus region of interest (ROI) using the Wake Forest University PickAtlas (Lancaster et al., 1997, Lancaster et al., 2000, Maldjian et al., 2003), and following (Shao et al. 2013, Shao et al, 2014, Lei et al, 2014]), we selected right and left thalamus ROI:s as well as separate ROI:s for the superficial, centromedial, and basolateral amygdala, as defined by Amunts et al., 2005 using the Jülich atlas (Eickhoff et al., 2005, Eickhoff et al., 2006, Eickhoff et al., 2007).

2.10 Regional homogeneity (ReHo)

Using the DPARSFA toolbox, ReHo was estimated in data preprocessed as described above but without smoothing, with cluster size of 27 voxels (3x3x3) and subsequent smoothing. 2nd level analysis was performed in SPM12 as described above.

2.11 Global signal

Global signal was estimated using the DPARSFA toolbox. For each run, global signal variability was determined as the standard deviation of gray matter signal during the run, following Wong et al. (2013, 2016). Notably, Wong et al. (2013, 2016) calculated the same measure but called it amplitude rather than variability, and we used that terminology in the registration of hypotheses. To better approximate a normal distribution, standard deviations were log-transformed. Mixed-effects models were then used in R (R Core Team, 2015) to investigate effects of sleep deprivation and age group, as well as correlations to putative covariates, with mean framewise displacement as a covariate of no interest in order to decrease the influence of head motion on estimates.

2.12 Amplitude of low-frequency fluctuations (ALFF)

Amplitude of low-frequency fluctuations (ALFF) and fractional amplitude of low-frequency fluctuations (fALFF) were analysed using the DPARSFA toolbox on preprocessed data after nuisance regression but before scrubbing, in order to preserve continuity of the time-series. For the same reason, the second run in each session was not included in these analyses, as these runs had volumes censored.

2.13 Availability of data and code

Structural and functional imaging data will be available at https://openfmri.org/dataset/ds000201/. Code for preprocessing and analysis, SPM results objects, and results tables are available at http://dx.doi.org/10.5281/zenodo.60450. Masks and seed regions are available for visualisation and download at http://neurovault.org/collections/LEWNNZLY/.

3 Results

3.1 Participants

The final sample consisted of 53 participants (30 young, 23 old), after excluding 15 due to excessive motion (8 young, 7 old). Participant characteristics are given in table 1. PSG data for the full sample of participants have been reported in Åkerstedt et al. (in prep), but are repeated here for those participants finally included in resting state analyses.

	Condition	All	Young	Old
Sex (F/M)		30/23	16/14	13/10
Age (median, range)		26 (20-75)	23 (20-29)	68 (65-75)
Total sleep time, minutes (mean, SD)	Full sleep	412 (76)	431 (78)	389 (68)
	Sleep deprivation	173 (37)	184 (37)	158 (33)
REM, % (mean, sd)	Full sleep	19.5 (6.9)	19.8 (5.6)	19.2 (8.3)
	Sleep deprivation	15.4 (8.8)	14.4 (6.9)	16.0 (10.0)
N1, % (mean, SD)	Full sleep	18.8 (10.1)	14.4 (6.6)	28.6 (11.1)
	Sleep deprivation	15.6 (9.0)	10.8 (5.8)	20.6 (9.4)
N2, % (mean, SD)	Full sleep	44.1 (7.7)	42.8 (7.7)	45.8 (7.5)
	Sleep deprivation	40.7 (11.8)	35.5 (9.6)	45.4 (12.0)
N3, % (mean, SD)	Full sleep	17.5 (9.6)	23.0 (6.8)	10.4 (8.0)
	Sleep deprivation	28.3 (16.0)	39.3 (9.5)	18.1 (14.3)
ESS (mean, SD)		8.2 (3.8)	7.4 (2.8)	9.2 (4.6)
ISI (mean, SD)		9.9 (2.0)	10.1 (2.2)	9.0 (1.3)
KSQ sleep quality index (mean, SD)		5.3 (0.5)	5.3 (0.5)	5.2 (0.5)
KSQ snoring symptom index (mean, SD)		5.7 (0.4)	5.6 (0.3)	5.5 (0.5)

Table 1: Participant characteristics and sleep measures.

3.2 Measures of sleep and sleepiness

KSS data for the full sample of participants have been reported in Åkerstedt et al. (in prep), but are repeated here for those participants finally included in resting state analyses. Effects of sleep deprivation, age group, and time in scanner on KSS ratings was investigated using a mixed-effects regression model. Sleep deprivation caused increased KSS (β = 1.88 [95% CI 1.73, 2.03], $p < 0.001$, figure 2). Young age group was associated to higher KSS ratings (1.33 [0.67, 2.00], $p < 0.001$), as was time in scanner (0.76 per hour [0.60, 0.92], $p < 0.001$, figure 2).

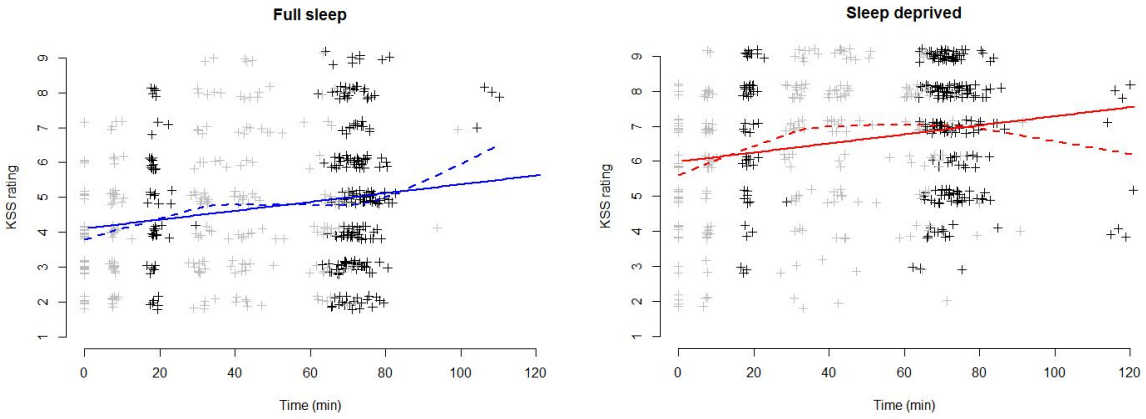

Figure 2: KSS ratings. Left: Full sleep condition. Right: Sleep deprivation condition. Data have been vertically jittered to aid visualisation. Points in black show KSS ratings made in connection to resting state experiments, and points in gray show other KSS ratings. Straight lines show linear regressions; dashed lines show loess regressions. In most cases, imaging was completed within 90 minutes and data points occurring later are mostly due to technical or other interruptions in the experiment, with participants exiting the MRI scanner and entering again.

3.3 Independent component analyses

As reported for the purpose of technical validation in Nilsonne et al. (2016), ICA analyses revealed 20 networks, of which 17 were judged to represent neural activity and 3 artifacts associated to head motion and large blood vessels. Of the 17 components representing neural activity, nine described default mode, frontoparietal attention, executive control, and salience networks, and were analysed further (figure 2).

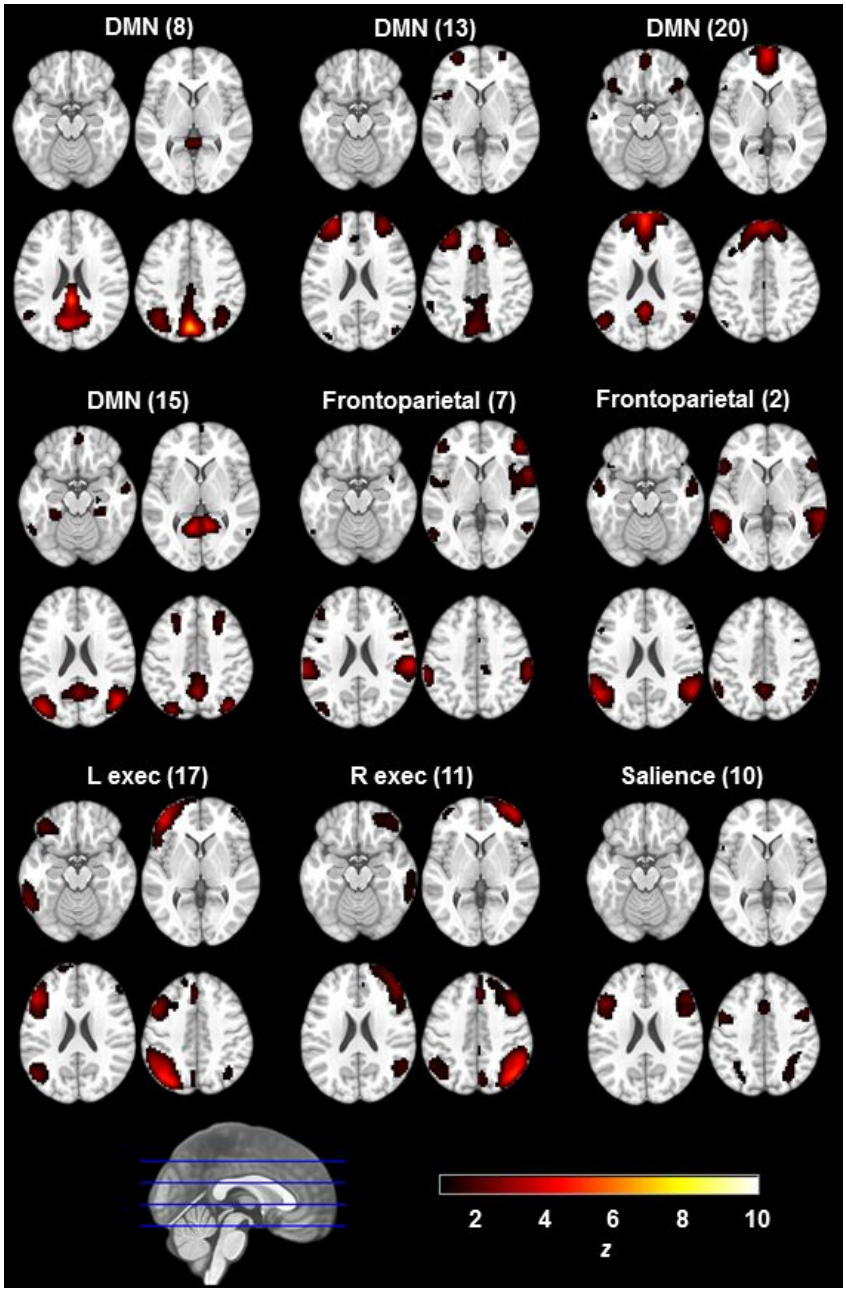

Figure 2: ICA networks of interest. Seventeen of 20 networks were judged to represent meaningful neurophysiological connectivity. Nine of these 17 included the default mode network (DMN), frontoparietal attention, executive control (lateralised left and right), and salience networks, and were examined further. Images are displayed in neurological convention.

Sleep deprivation did not cause changes in connectivity within default mode, executive control, and salience networks (significance level $p_{FWE} < 0.05$). Younger participants showed a pattern of greater connectivity than older participants within most networks (figure 3). Older participants showed higher connectivity in small scattered foci (not shown; available at

http://dx.doi.org/10.5281/zenodo.60450). Sleep deprivation and age group showed no significant interactions.

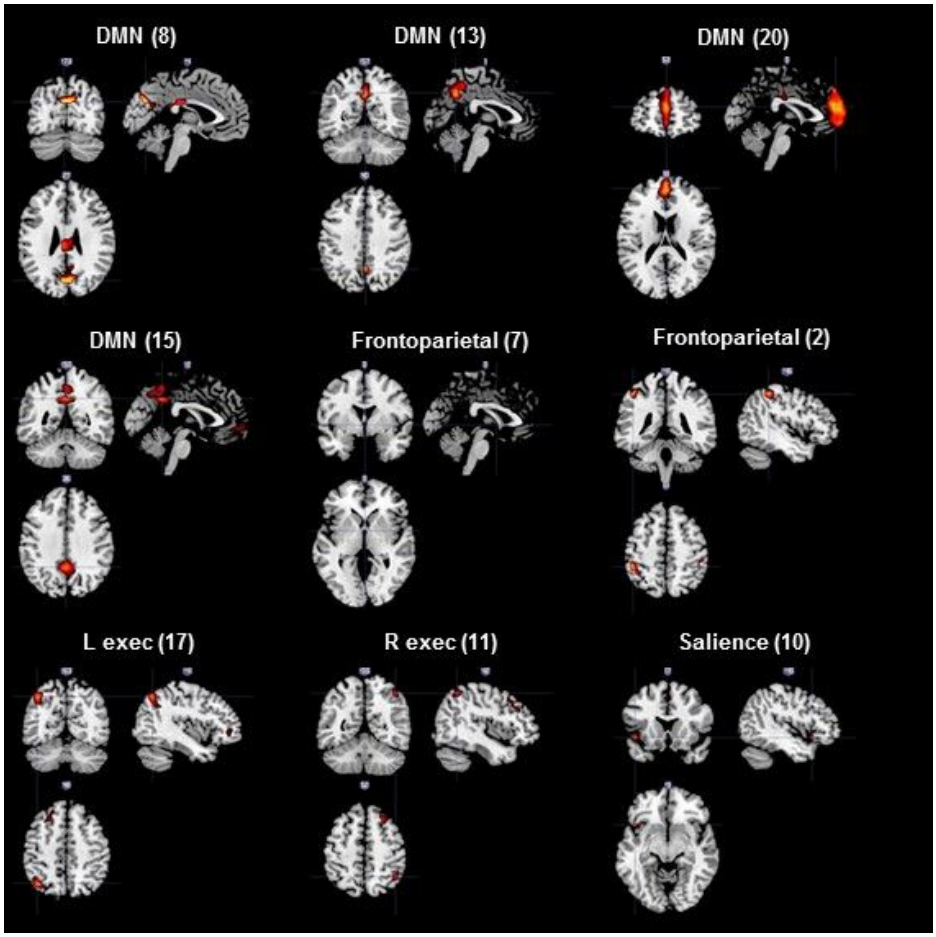

Figure 3: ICA results. Areas within network masks where younger participants showed higher functional connectivity compared to older participants, in 8 of the 9 networks (p_{FWE} < 0.05. Component 7 (frontoparietal) showed no significant differences).

3.4 Seed-based analyses of default mode and task-positive networks

Besides the data-driven ICA approach, we also tested a specific hypothesis-driven set of correlations, namely between the default mode network (DMN) and the task-positive network (TPN). We used seed regions defined in a previous paper on sleep deprivation (De Havas et al., 2012, figure 4).

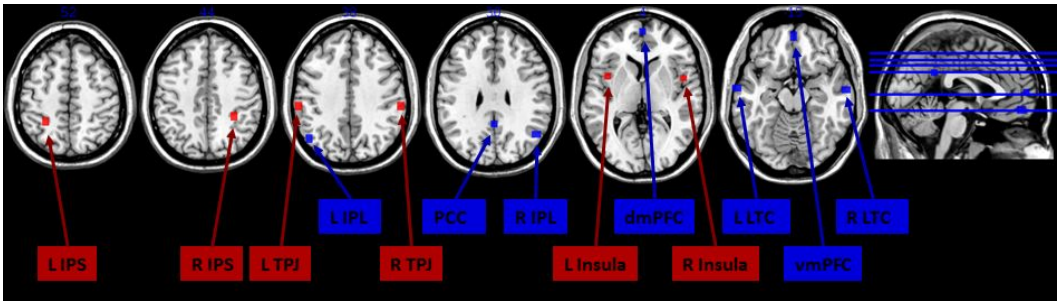

Figure 4: Seed regions for the default mode network (DMN, blue) and task-positive network (TPN, red), defined from de Havas et al, 2012. IPL: inferior parietal lobule. IPS: inferior parietal sulcus. LTC: Lower temporal cortex. PCC: posterior cingulate cortex. dmPFC: dorsomedial prefrontal cortex. vmPFC: ventromedial prefrontal cortex. TPJ: temporo-parietal junction.

An overall pattern of correlation between DMN pairs and anticorrelation between DMN nodes and ACN nodes was confirmed (figure 5). Sleep deprivation caused no significant changes surviving multiple comparisons correction at $p < 0.05$. Younger participants showed a pattern of higher connectivity within the DMN, with one node pair (right and left lower temporal cortex).

Total sleep time was associated with increased connectivity between RIPL and LLTC in the interaction between older group and sleep deprivation (0.027, $p_{FDR} = 0.04$). None of the other putative sleep-related covariates listed in the hypotheses (above) correlated significantly to ROI-based connectivity measures at $p < 0.05$ after false discovery rate correction for multiple testing within each contrast (main effect, interaction with sleep condition, interaction with age group, and 3-way interaction).

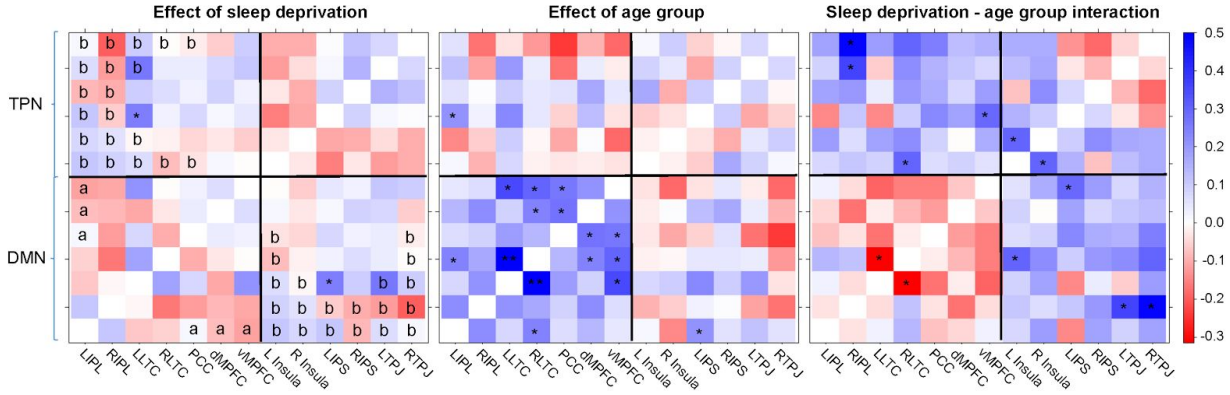

Figure 5: Connectivity results between regions of interest in the default mode network and the task-positive network. The effects of modelled predictors (sleep deprived vs full sleep, older vs younger group, and their interaction) is shown on pair-wise association between roi time courses. For abbreviations, see legend to fig. 4. a: Decreased correlation observed by de Havas et al. (predicted effect is negative; red). b: Decreased anticorrelation observed by de Havas et al. (predicted effect is positive; blue). * $p < 0.05$

uncorrected. ** $p < 0.05$ FDR-corrected. Note that figures are symmetrical about the diagonal.

3.5 Seed-based analyses of connectivity with thalamus and amygdala

Based on reports of effects of sleep deprivation on connectivity from the thalamus (Yeo et al., 2015, Shao et al., 2013) and amygdala (Shao et al., 2014, Lei et al., 2015), we investigated whether earlier results could be replicated. Seed regions are shown in supplementary figures 2 and 4 for thalamus and amygdala, respectively. Functional connectivity with the thalamus was exhibited in the anterior and posterior cingulate cortices, the occipital cortex, and the cerebellum (supplemental figure 3). Functional connectivity with bilateral amygdalae was shown in large parts of the brain, including contralateral amygdala, basal ganglia, and cortical areas in all four lobes (supplemental figure 5). Sleep deprivation caused no differences in connectivity with thalamus or amygdala seeds exceeding a threshold of $p_{FWE} = 0.05$, nor did any differences between the age groups exceed that threshold.

3.6 Regional homogeneity (ReHo)

Sleep deprivation did not affect measures of ReHo. Younger participants had higher ReHo in areas of the cerebral cortex and basal ganglia, particularly in the medial prefrontal cortex and in the superior temporal cortex/insula bilaterally (figure 6). Older participants had higher ReHo mainly in areas prone to imaging and motion artifacts, including the orbitofrontal cortex and the outer edges of the brain anteriorly and posteriorly. Therefore, and even though framewise displacement was included as a 2nd-level regressor of no interest to account for motion artifacts, areas with apparently higher ReHo among the elderly may not reflect genuine differences in homogeneity of cerebral blood flow. There was no significant interaction between age group and sleep condition.

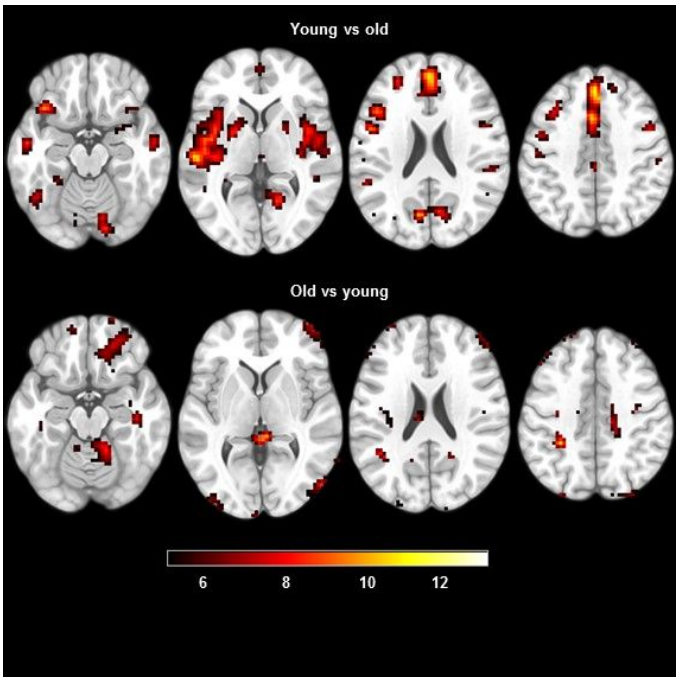

Figure 6: Differences in regional homogeneity between younger and older participants.

3.7 Global signal

Global signal was not used as a covariate in our analyses reported above, since global signal regression has been identified as a potential source of spurious correlations (Murphy et al. 2009, Saad et al. 2012). However, we separately investigated whether sleep deprivation affected the global signal. Our main outcome of interest was global signal variability.

We found that sleep deprivation caused higher global signal variability (0.16 [0.07, 0.24], p = 0.0004, figure 7). Global signal variability was lower in older participants (-0.33 [-0.50, -0.16], p = 0.0002). The interaction between sleep deprivation and older age was -0.11 [-0.24, 0.02], p = 0.09. We found no strong associations between 15 putative predictors and global signal variability (supplemental table 2). In some cases, p-values < 0.05 and >0.01 were observed for interaction effects between conditions, but as these effects were not significant at alpha < 0.05 in either condition, we are reluctant to interpret them as positive associations. The exception is wake after sleep onset (WASO), which in the full sleep condition was associated with lower global signal variability (p = 0.03), and especially in the younger group (p = 0.03). As increased wake after sleep onset suggests worse sleep quality, this finding is in the contrary direction compared to the main effect.

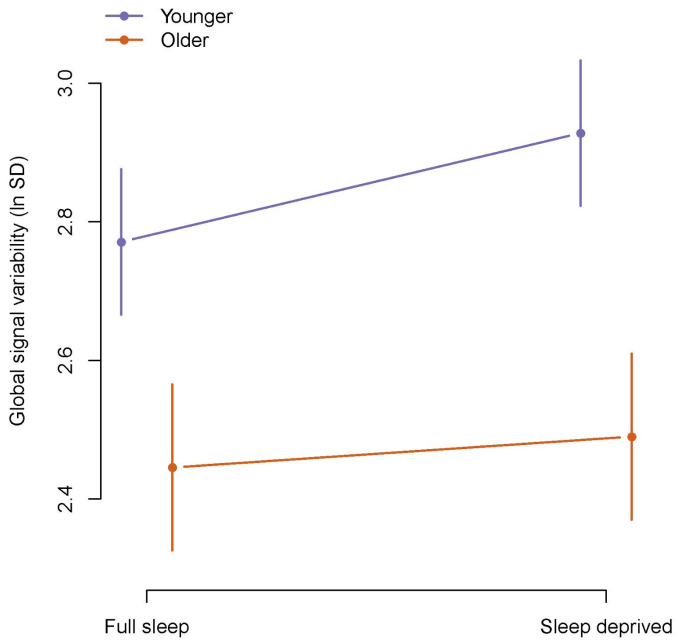

Figure 7: Global signal variability (standard deviation, transformed using the natural logarithm) after sleep deprivation in younger and older participants. Error bars show 95% confidence intervals.

Effects of sleep deprivation and age group on the global signal itself, rather than its variability, are shown in supplemental figure 6. Sleep deprivation and age group interacted with higher global signal in the older group after sleep deprivation (106.0 [46.0, 166.0], $p = 0.0006$). There was a main effect of sleep deprivation, causing lower global signal (-68.1 [-107.7, -28.6], $p = 0.009$). The main effect of age group was not statistically significant (-8.7 [-143.8, 126.3], $p = 0.90$). We found no strong associations between 15 putative predictors and global signal (supplemental table 3).

3.7 Amplitude of low-frequency fluctuations

ALFF and fALFF were investigated exploratively as differences in ALFF following sleep deprivation were shown in a recent publication (Wang et al., 2016). We found no significant differences between sleep conditions exceeding a threshold of $p_{FWE} > 0.05$, and only scattered foci in comparisons between age groups (results not shown; available at [NeuroVault]).

4 Discussion

We found that partial sleep deprivation caused higher global signal variability. We did not find other major effects of partial sleep deprivation on measures of intrinsic connectivity, including ICA-derived networks; seed-based connectivity in the DMN and TPN and from the thalamus and amygdala to the rest of the brain; ALFF; and ReHo. Older participants generally showed less functional connectivity than younger participants. Major interactions between age group and sleep deprivation were not observed.

It is possible that global signal variability increased in sleepiness because of the increased propensity to drift in and out of sleep (wake-state instability), as the transition to sleep is associated with changes in network connectivity (Tagliazucchi et al., 2013). Our findings agree well with the earlier findings that global signal variability is decreased by caffeine (Wong et al. 2012) and that global signal variability correlates to EEG measures of vigilance (Wong et al, 2013 and 2016). This finding is also consistent with findings by Fukunaga et al. (2006) that global signal variability increased in sleep, and by Kiviniemi et al. (2005) that midazolam sedation caused increased global signal variability.

In line with previous research (Sala-Llonch et al., 2015) older participants had lower connectivity within most ICA-derived networks of interest. Furthermore, we found that older participants had lower regional homogeneity (ReHo) in medial prefrontal cortex as well as superior temporal lobes and insula bilaterally. To our knowledge, only one study has previously investigated the effect of normal aging on ReHo in the resting brain, finding lower ReHo in motor areas (Wu et al., 2007).

In the present study, we used a partial sleep deprivation paradigm, mainly because it has higher ecological validity compared with total sleep deprivation. Increased subjective sleepiness in the PSD condition confirms that the current protocol successfully induced sleepiness. The KSS measure of subjective sleepiness has been closely related to behavioral and physiological sleepiness (Åkerstedt et al., 2014). KSS levels reached after PSD correspond to those seen

during night work or night driving, although not as high as that seen before driving off the road in a driving simulator or being taken off the road for sleep related dangerous driving (Åkerstedt et al., 2014). Thus, it is possible that the sleep manipulation might not have been strong enough to cause alterations in intrinsic connectivity measures, for which we found no effects. Thus, one possible conclusion is that partial sleep deprivation and the associated moderate sleepiness may not cause changes in intrinsic connectivity.

Another possible explanation may be that functional brain imaging following sleep deprivation may be confounded by intrusions of sleep during the experiment (Tagliazucchi and Laufs, 2014). In this case, effects attributed to sleepiness in previous studies may in fact be due to sleep. In this study, we used partial sleep deprivation, resting state data were acquired with eyes open, and participants were monitored using eye-tracking. These features reduced the risk of sleep intrusions, offering one possible explanation for the non-replication of some previously reported effects.

Limitations include head motion, which was higher after sleep deprivation (Nilsonne et al. 2016), and which is expected to cause an apparent decrease in long-range connectivity, e.g. within the default mode network (Power et al., 2013), and an increase in short-range connectivity, e.g. regional homogeneity. Strengths of this study include a relatively large sample that includes both younger and older adults , recording of PSG and KSS, and monitoring of participants by eye-tracking.

In conclusion, we report that global signal variability was increased by sleep deprivation. Although the global signal remains poorly understood, and it is not known to which extent it represents neural activity as opposed to e.g. vascular auto-regulation, it is possible that the global signal variability may represent a biomarker for sleepiness. This finding should be replicated in independent data.

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Authors' contributions
Designed the study: GN, ST, JS, HF, GK, ML, PF, TÅ. Acquired data: GN, ST. Analysed data: GN, RA, PF. Interpreted results: GN, ST, JS, RA, HF, GK, ML, PF, TÅ. Drafted manuscript: GN. All authors read and approved the final version of the manuscript.

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Supplemental table 1

Coordinates for seed regions based on de Havas et al. 2012.

		Talairach			MNI		
		x	y	z	x	y	z
DMN	PCC	1	-50	26	1	-29	49
	dmPFC	0	53	16	1	59	7
	vmPFC	0	50	-4	1	54	-14
	L IPL	-45	-66	31	-47	-66	37
	R IPL	45	-61	25	50	-61	29
	L LTC	-62	-7	-8	-66	-7	-12
	R LTC	57	-9	-14	62	-9	-20
ACN	L Insula	-39	5	7	-41	7	3
	R Insula	44	2	10	49	5	5
	L TPJ	-57	-31	34	-60	-28	37
	R TPJ	54	-31	34	60	-28	35
	L IPS	-27	-49	49	-27	-46	55
	R IPS	27	-43	43	31	-40	47

Supplemental table 2

Effects of KSS and sleep parameters on global signal variability in full sleep and sleep deprivation conditions. Because the sleep measures were so different between the full sleep and sleep deprived condition, regression models including both conditions are prone to unreliable results due to linear separation. Therefore, all covariates have been analysed separately in each condition, as well as in a full model including both conditions. Thus, the first column (Full sleep) reports the effect of each predictor in the full sleep condition only. The second column (Sleep deprived) reports the effect of each predictor in the sleep deprived condition only, and the last column (Interaction) reports the difference between conditions in the relation between each predictor and global signal variability. The table reports main effects as well as age group interaction effects, indicating estimated differences between older and younger participants in the relation of each predictor to the global signal. Thus, for example, the "KSS-age group interaction" row and the "Interaction" column give the estimated effect of increasing 1 step on the KSS scale and moving from the younger to the older group. Estimates are on the original scale, as illustrated in figure 7. For main effects of age group, see main text.

	Full sleep		**Sleep deprived**		**Interaction**	
	Estimate [CI]	***p***	**Estimate [CI]**	***p***	**Estimate [CI]**	***p***
KSS main effect	-0.011 [-0.06, 0.038]	0.66	0.043 [-0.018, 0.105]	0.16	0.036 [-0.003, 0.074]	0.07
KSS-age group interaction	0.006 [-0.071, 0.082]	0.88	-0.076 [-0.166, 0.014]	0.10	-0.043 [-0.089, 0.003]	0.06
TST main effect	0.000 [-0.001, 0.002]	0.81	-0.006 [-0.013, 0.000]	0.05	0.000 [-0.001, 0.001]	0.45
TST-age group interaction	0.000 [-0.002, 0.003]	0.73	0.002 [-0.006, 0.01]	0.57	0.001 [0.000, 0.002]	0.00
REM% main effect	-0.002 [-0.025, 0.02]	0.84	-0.005 [-0.016, 0.007]	0.43	-0.001 [-0.004, 0.002]	0.44
REM%-age group interaction	0.01 [-0.019, 0.039]	0.49	0.002 [-0.012, 0.016]	0.80	0.003 [0.000, 0.005]	0.02
N1% main effect	-0.016 [-0.033, 0.002]	0.08	0.002 [-0.024, 0.028]	0.86	-0.017 [-0.03, -0.003]	0.01
N1%-age group interaction	0.012 [-0.01, 0.035]	0.27	0.009 [-0.021, 0.039]	0.54	0.015 [-0.001, 0.03]	0.06
N2% main effect	0.006 [-0.008, 0.021]	0.38	0.002 [-0.01, 0.015]	0.70	0.004 [-0.007, 0.014]	0.46
N2%-age group interaction	-0.014 [-0.041, 0.014]	0.32	-0.004 [-0.02, 0.013]	0.67	-0.003 [-0.014, 0.008]	0.61
N3% main effect	0.000 [-0.017, 0.017]	1.00	-0.002 [-0.015, 0.011]	0.77	0.001 [-0.009, 0.012]	0.80
N3%-age group interaction	0.000 [-0.024, 0.025]	0.99	-0.001 [-0.017, 0.015]	0.87	-0.003 [-0.012, 0.005]	0.48

Stage changes main effect	-0.012 [-0.03, 0.006]	0.18	0.014 [-0.008, 0.035]	0.21	-0.009 [-0.022, 0.005]	0.20
Stage changes-age group interaction	-0.004 [-0.029, 0.02]	0.73	-0.011 [-0.038, 0.016]	0.40	-0.005 [-0.021, 0.012]	0.58
Delta power (absolute) main effect	0.028 [-0.198, 0.253]	0.80	-0.068 [-0.279, 0.144]	0.52	-0.084 [-0.245, 0.077]	0.30
Delta power (absolute)-age group interaction	-0.004 [-0.575, 0.568]	0.99	0.271 [-0.131, 0.674]	0.18	0.137 [-0.178, 0.452]	0.39
Delta power (relative) main effect	0.898 [-2.919, 1.123]	0.37	-1.08 [-2.977, 0.817]	0.26	-1.039 [-2.434, 0.355]	0.14
Delta power (relative)-age group interaction	1.061 [-2.443, 4.566]	0.54	0.478 [-2.186, 3.141]	0.72	0.861 [-1.067, 2.788]	0.38
Number of awakenings main effect	-0.016 [-0.032, 0.001]	0.06	-0.014 [-0.061, 0.034]	0.57	-0.016 [-0.028, -0.004]	0.01
Number of awakenings-age group interaction	0.01 [-0.012, 0.033]	0.36	0.03 [-0.03, 0.09]	0.32	0.016 [0.004, 0.029]	0.01
Sleep efficiency main effect	0.021 [-0.006, 0.049]	0.12	0.028 [-0.007, 0.063]	0.11	0.015 [-0.005, 0.036]	0.14
Sleep efficiency-age group interaction	-0.022 [-0.054, 0.01]	0.18	-0.035 [-0.072, 0.001]	0.06	-0.024 [-0.045, -0.003]	0.03
Wake after sleep onset main effect	-0.008 [-0.014, -0.001]	0.03	-0.014 [-0.031, 0.003]	0.11	-0.007 [-0.012, -0.002]	0.01
Wake after sleep onset-age group interaction	0.008 [0.001, 0.016]	0.03	0.016 [-0.005, 0.037]	0.13	0.008 [0.003, 0.013]	0.002
ISI main effect	-0.002 [-0.056, 0.051]	0.93	-0.045 [0.003, 0.052]	0.89	0.013 [-0.018, 0.046]	0.40
ISI-age group interaction	0.015 [-0.101, 0.131]	0.79	-0.000 [-0.105, 0.104]	1.00	0.009 [-0.089, 0.107]	0.85
ESS main effect	0.013 [-0.029, 0.056]	0.53	-0.002 [-0.041, 0.036]	0.91	0.010 [-0.026, 0.047]	0.31
ESS-age group interaction	-0.003 [-0.054, 0.049]	0.92	0.012 [-0.035, 0.058]	0.62	0.004 [-0.039, 0.048]	0.85
KSQ sleep quality main effect	0.091 [-0.167, 0.350]	0.48	0.022 [-0.213, 0.256]	0.85	-0.034 [-0.178, 0.110]	0.64
KSQ sleep quality-age group interaction	-0.108 [-0.500, 0.285]	0.58	-0.025 [-0.381, 0.330]	0.89	-0.068 [-0.400, 0.263]	0.68

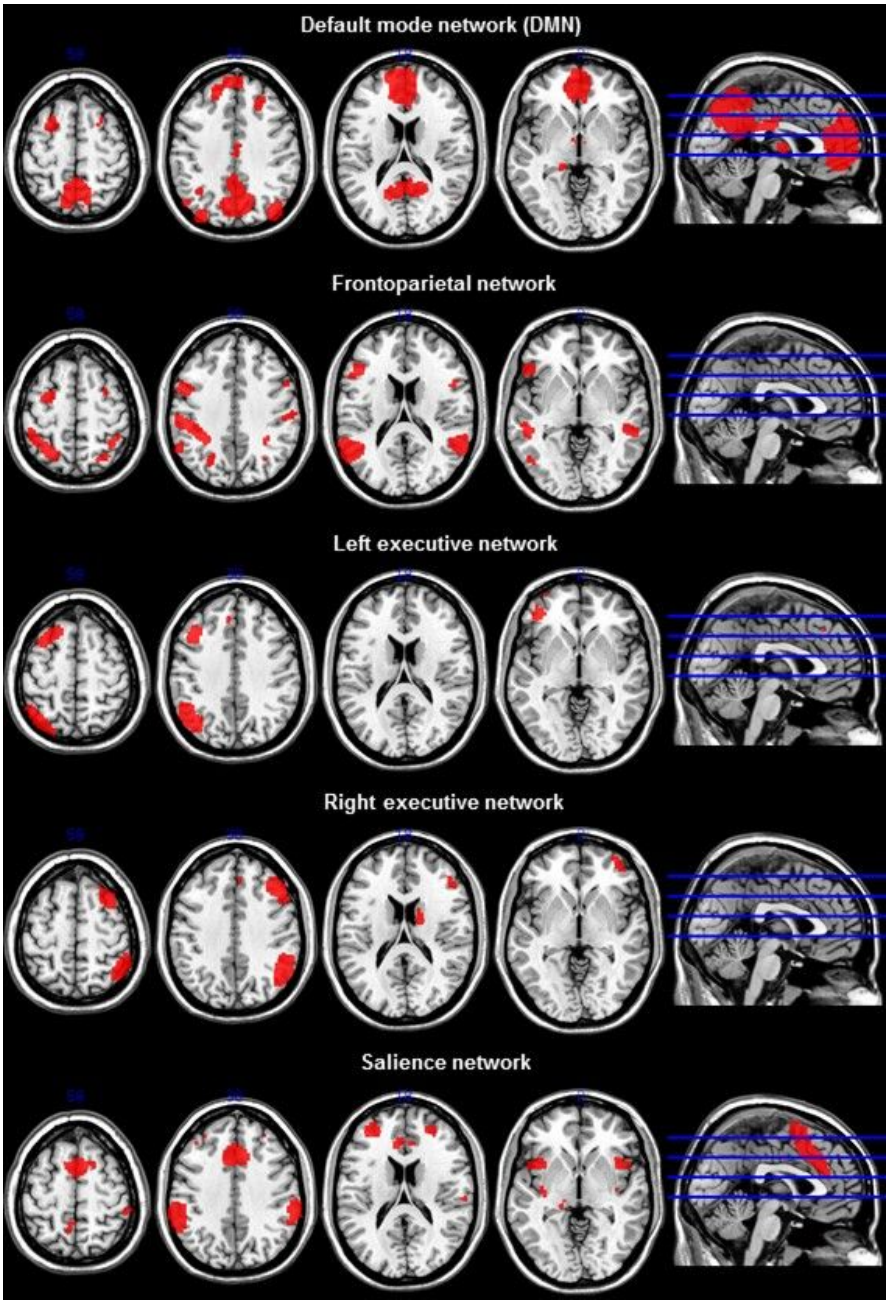
Supplemental table 3

Effects of KSS and sleep parameters on global signal in full sleep and sleep deprivation conditions. Estimates are on the original scale, as illustrated in supplemental figure 6.

	Full sleep		**Sleep deprived**		**Interaction**	
	Estimate [CI]	***p***	**Estimate [CI]**	***p***	**Estimate [CI]**	***p***
KSS main effect	-1.416 [-21.932, 19.1]	0.89	8.96 [-10.486, 28.406]	0.36	-1.099 [-19.958, 17.76]	0.91
KSS-age group interaction	24.871 [-7.371, 57.114]	0.13	-4.06 [-32.935, 24.816]	0.78	20.034 [-2.767, 42.836]	0.08
TST main effect	-0.246 [-1.734, 1.242]	0.74	-2.542 [-8.553, 3.469]	0.40	0.472 [-0.014, 0.959]	0.06
TST-age group interaction	0.919 [-1.962, 3.801]	0.52	4.162 [-3.265, 11.589]	0.26	0.117 [-0.235, 0.469]	0.51
REM% main effect	-5.617 [-28.469, 17.236]	0.62	-7.912 [-28.144, 12.319]	0.43	-1.161 [-9.81, 7.487]	0.79
REM%-age group interaction	1.813 [-27.712, 31.338]	0.90	5.053 [-18.064, 28.169]	0.66	-4.499 [-12.109, 3.11]	0.24
N1% main effect	-0.002 [-16.5, 16.495]	1.00	-7.422 [-28.976, 14.132]	0.49	-0.001 [-7.004, 7.002]	1.00
N1%-age group interaction	-6.154 [-27.216, 14.908]	0.56	6.008 [-19.17, 31.186]	0.63	-1.916 [-10.411, 6.579]	0.66
N2% main effect	0.933 [-12.359, 14.224]	0.89	-0.065 [-10.359, 10.229]	0.99	1.898 [-3.424, 7.219]	0.48
N2%-age group interaction	-8.618 [-33.991, 16.756]	0.50	-4.287 [-17.992, 9.419]	0.53	0.097 [-5.043, 5.236]	0.97
N3% main effect	0.028 [-0.198, 0.253]	0.80	3.463 [-6.695, 13.62]	0.50	3.618 [-1.248, 8.485]	0.14
N3%-age group interaction	-0.004 [-0.575, 0.568]	0.99	1.598 [-11.007, 14.203]	0.80	1.290 [-2.601, 5.181]	0.51
Stage changes main effect	-0.434 [-17.809, 16.942]	0.96	-10.02 [-27.935, 7.895]	0.27	-4.706 [-11.53, 2.118]	0.17
Stage changes-age group interaction	-9.507 [-32.889, 13.876]	0.42	12.909 [-9.399, 35.218]	0.25	0.522 [-8.306, 9.349]	0.91

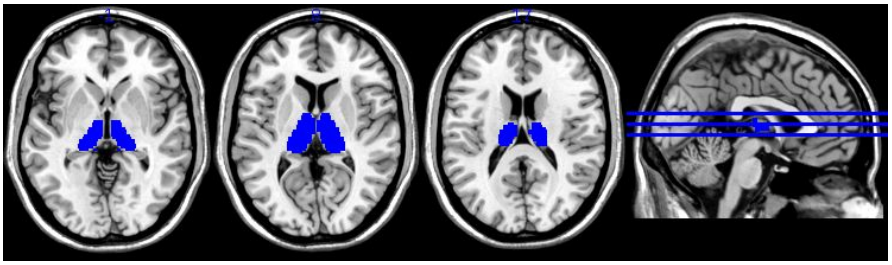
Delta power (absolute) main effect	0.028 [-0.198, 0.253]	0.80	-0.068 [-0.279, 0.144]	0.52	-0.084 [-0.245, 0.077]	0.30
Delta power (absolute)-age group interaction	-0.004 [-0.575, 0.568]	0.99	0.271 [-0.131, 0.674]	0.18	0.137 [-0.178, 0.452]	0.39
Delta power (relative) main effect	-0.898 [-2.919, 1.123]	0.37	-1.08 [-2.977, 0.817]	0.26	-1.039 [-2.434, 0.355]	0.14
Delta power (relative)-age group interaction	1.061 [-2.443, 4.566]	0.54	0.478 [-2.186, 3.141]	0.72	0.861 [-1.067, 2.788]	0.38
Number of awakenings main effect	-3.346 [-18.831, 12.139]	0.67	-11.614 [-50.94, 27.713]	0.55	0.12 [-5.298, 5.538]	0.96
Number of awakenings-age group interaction	-6.252 [-27.328, 14.825]	0.55	14.064 [-35.274, 63.403]	0.57	-4.11 [-9.75, 1.53]	0.15
Sleep efficiency main effect	-1.531 [-27.173, 24.111]	0.90	29.617 [1.045, 58.19]	0.04	-11.234 [-21.632, -0.836]	0.03
Sleep efficiency-age group interaction	8.439 [-21.558, 38.435]	0.57	-30.541 [-60.611, -0.47]	0.05	15.943 [5.317, 26.57]	0.00
Wake after sleep onset main effect	-0.975 [-7.441, 5.491]	0.76	-15.119 [-28.959, -1.279]	0.03	2.636 [0.377, 4.895]	0.02
Wake after sleep onset-age group interaction	-0.165 [-7.457, 7.128]	0.96	14.15 [-2.713, 31.012]	0.10	-3.639 [-5.791, -1.488]	0.00
ISI main effect	-3.340 [-49.380, 42.700]	0.88	-0.079 [-39.588, 39.430]	1.00	4.762 [-36.139, 45.663]	0.82
ISI-age group interaction	-9.004 [-108.194, 90.186]	0.86	-24.389 [-109.511, 60.734]	0.57	-11.677 [-98.227, 74.872]	0.79
ESS main effect	-20.180 [-56.429, 16.069]	0.27	-27.295 [-57.903, 3.313]	0.08	-22.341 [-53.994, 9.312]	0.16
ESS-age group interaction	24.678 [-19.433, 68.789]	0.27	30.720 [-6.530, 67.970]	0.10	26.266 [-11.913, 64.445]	0.17
KSQ sleep quality main effect	75.301 [-146.347, 296.948]	0.50	106.850 [-82.004, 295.705]	0.26	79.934 [-115.583, 275.45]	0.42
KSQ sleep quality-age group interaction	-116.908 [-453.692, 219.876]	0.49	-168.563 [-455.595, 118.469]	0.24	-145.014 [-437.483, 147.454]	0.32

Supplemental figure 1: Masks for resting state networks of interest



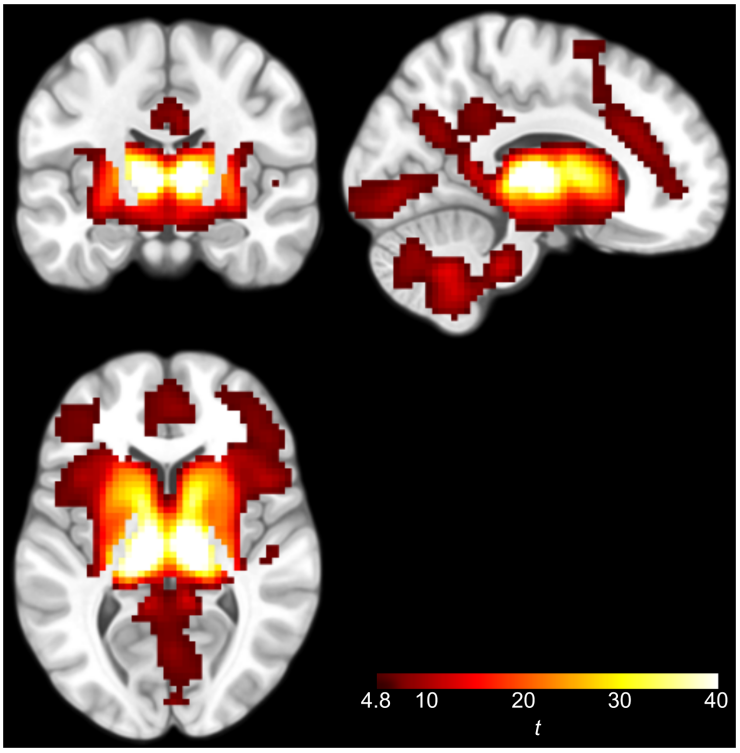
Networks were defined based on a parcellation published previously by Shirer et al. (2012).

Supplemental figure 2: Thalamus seed region



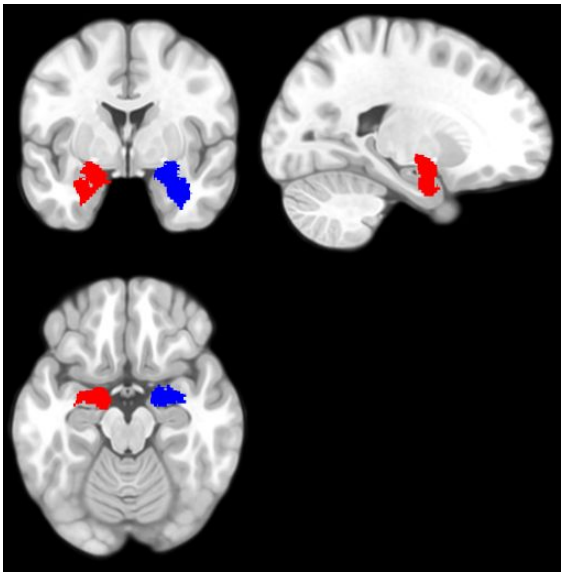
Seed region for bilateral thalamus, defined from the Wake Forest University PickAtlas, following Shao et al., 2013.

Supplemental figure 3: Thalamus connectivity



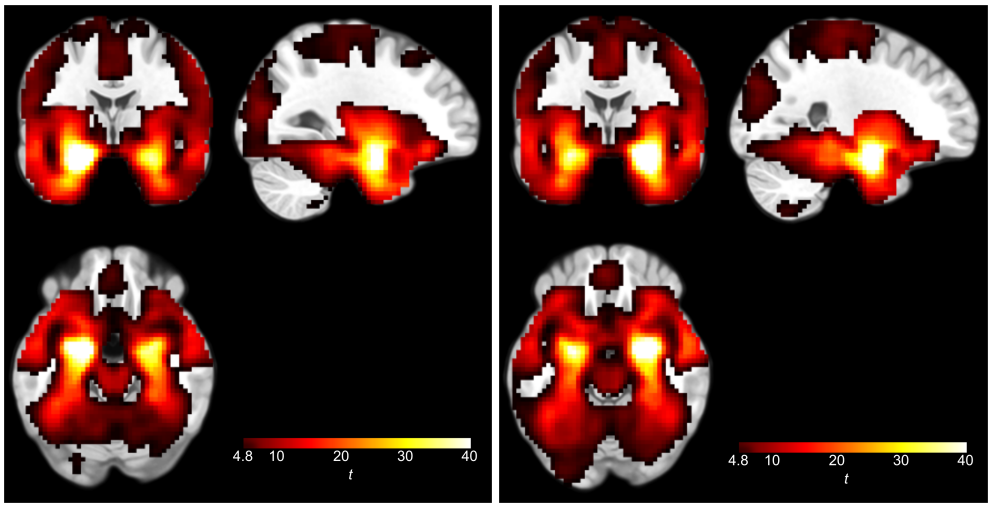
Thalamus connectivity across conditions and groups. Scale truncated at $t = 40$.

Supplemental figure 4: Amygdala seed regions



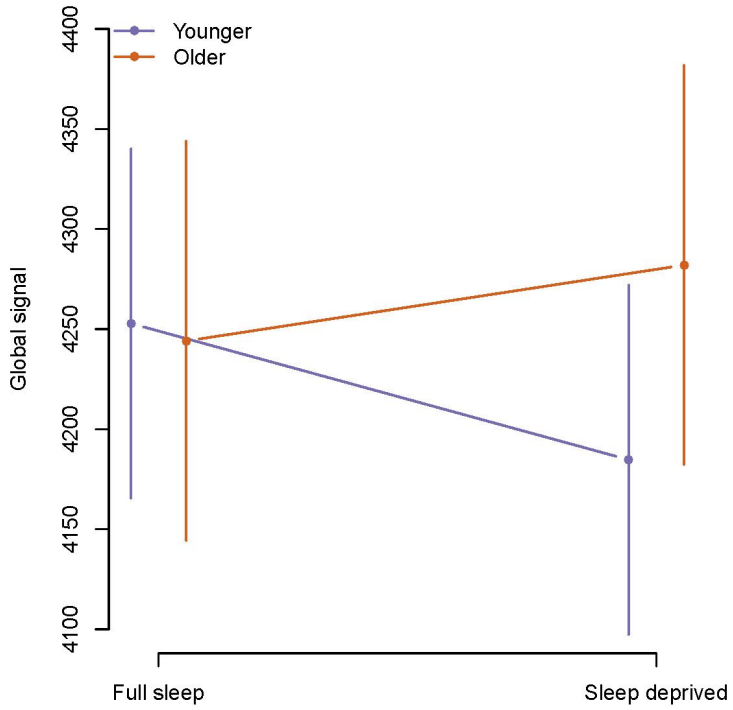
Seed regions for left and right amygdala, defined from the Jülich Atlas.

Supplemental figure 5: Amygdala connectivity



Left and right amygdala connectivity across conditions and groups. Scale truncated at t = 40.

Supplemental figure 6: Global signal



Global signal after sleep deprivation in younger and older participants. Error bars show 95% confidence intervals.