NeuroImage 184 (2019) 266–278

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/neuroimage

Hard to wake up? The cerebral correlates of sleep inertia assessed using combined behavioral, EEG and fMRI measures

Raphael Vallat *, David Meunier, Alain Nicolas, Perrine Ruby

DYCOG Team, Lyon Neuroscience Research Center, CNRS UMR 5292, INSERM U1028, Lyon 1 University, France

ARTICLE INFO

Keywords:
Awakening
Default mode network
NREM sleep
Sleep deprivation
Nap
Functional connectivity

ABSTRACT

The first minutes following awakening from sleep are typically marked by reduced vigilance, increased sleepiness and impaired performance, a state referred to as sleep inertia. Although the behavioral aspects of sleep inertia are well documented, its cerebral correlates remain poorly understood. The present study aimed at filling this gap by measuring in 34 participants the changes in behavioral performance (descending subtraction task, DST), EEG spectral power, and resting-state fMRI functional connectivity across three time points: before an early-afternoon 45-min nap, 5 min after awakening from the nap and 25 min after awakening. Our results showed impaired performance at the DST at awakening and an intrusion of sleep-specific features (spectral power and functional connectivity) into wakefulness brain activity, the intensity of which was dependent on the prior sleep duration and depth for the functional connectivity (14 participants awakened from N2 sleep, 20 from N3 sleep). Awakening in N3 (deep) sleep induced the most robust changes and was characterized by a global loss of brain functional segregation between task-positive (dorsal attention, salience, sensorimotor) and task-negative (default mode) networks. Significant correlations were observed notably between the EEG delta power and the functional connectivity between the default and dorsal attention networks, as well as between the percentage of mistake at the DST and the default network functional connectivity. These results highlight (1) significant correlations between EEG and fMRI functional connectivity measures, (2) significant correlations between the behavioral aspect of sleep inertia and measures of the cerebral functioning at awakening (both EEG and fMRI), and (3) the important difference in the cerebral underpinnings of sleep inertia at awakening from N2 and N3 sleep.

1. Introduction

Sleep inertia is defined as the transient physiological state occurring just after awakening from sleep, which is characterized by reduced vigilance, increased sleepiness and impaired cognitive and physical performances (Tassi and Muzet, 2000; Trotti, 2017). As Lynn Trotti clearly pointed out in the title of her review *"waking up is the hardest thing I do all day"* (Trotti, 2017), sleep inertia is also usually experienced as unpleasant. Although its duration is not consistent and varies depending on the outcome measure used it is generally admitted that most of the behavioral effects of sleep inertia dissipate progressively in the first 30 min (min) post-awakening. The severity of sleep inertia has been positively associated with several factors such as prior sleep deprivation, awakening near the circadian trough of body temperature, awakening in N3 (deep, or slow-wave) sleep and some sleep disorders (Tassi and Muzet, 2000). Excessive sleep inertia, sometimes referred to as sleep drunkenness, is indeed a core feature of idiopathic hypersomnia and a component of delayed sleep phase disorder and non-rapid eye movement (NREM) sleep arousal parasomnias (Trotti, 2017).

A better understanding of sleep inertia is needed for the development of new strategies to reduce its detrimental effect on cognitive and physical performances, in pathological or physiological contexts alike. Sleep inertia may indeed have critical consequences in emergency situations when individuals are required to make vital decisions or actions immediately upon awakening (e.g. medical staff, firemen, pilots, military; Bruck and Pisani, 1999; Horne and Moseley, 2011). In the general population, sleep inertia represents the main limiting factor to the numerous beneficial effect of daytime napping (Faraut et al., 2017).

Abbreviations: DAN, dorsal attention network; DMN, default mode network; DST, descending subtraction task; EEG, electroencephalography; FDR, false discovery rate; fMRI, functional magnetic resonance imaging; FP, frontoparietal control network; HF, hippocampal formation; NREM, non rapid eye movement; PET, positron emission tomography; PSD, power spectral density; rCBF, regional cerebral blood flow; REM, rapid eye movement; SM, sensorimotor.

* Corresponding author. Center for Human Sleep Science, Department of Psychology, U.C Berkeley, USA.
E-mail address: raphaelvallat@berkeley.edu (R. Vallat).

https://doi.org/10.1016/j.neuroimage.2018.09.033
Received 3 July 2018; Received in revised form 21 August 2018; Accepted 12 September 2018
Available online 14 September 2018

The behavioral aspects of sleep inertia are well documented, but only a limited amount of studies have investigated its cerebral correlates until now. Using electroencephalography (EEG), some studies have found a persistence of slow activity (1–9 Hz) in the minutes following awakening, notably in posterior areas, a phenomenon which has been suggested to represent the electrophysiological signature of sleep inertia (Ferrara et al., 2006; Gorgoni et al., 2015; Marzano et al., 2011; Ogilvie and Simons, 1992). Using positron emission tomography (PET), Balkin and colleagues reported that the brain areas whose regional cerebral blood flow (rCBF) was increasing between 5 and 20 min post-awakening were primarily anterior heteromodal areas, such as the lateral prefrontal cortices and anterior insula (Balkin et al., 2002). They also reported shifts in the relative levels of rCBF between pairs of brain regions between 5 and 20 min post-awakening, leading them to propose that recovery from sleep inertia could hinge on a resumption of normal levels of both rCBF and functional connectivity between brain areas. The latter hypothesis has been tested in two recent studies which investigated the variations in brain networks functional connectivity between pre-sleep wakefulness, nocturnal sleep (without prior sleep deprivation) and post-sleep wakefulness (Tsai et al., 2014; Wu et al., 2012). Using paired comparisons between pre- and post-sleep wakefulness, they found a decreased connectivity within the sensory-motor (SM) network at awakening, but no alterations in cognitive networks. This altered connectivity within the SM network is consistent with the poor motor performances and clumsiness observed at awakening, but does not explain the cognitive impairments reported in previous studies (e.g. mental calculation; Tassi and Muzet, 2000).

Specifically, some modifications of the default mode network (DMN) connectivity could be expected at awakening since several studies showed consistent alterations of the DMN connectivity during sleep, fatigue and falling asleep (Picchioni et al., 2013). During N1 and N2 sleep, several teams found a decrease in the anti-correlation between the DMN and task-positive networks such as the dorsal attention network (DAN) and executive frontoparietal network (FP). This decreased anti-correlation has also been observed during wake after partial or total sleep deprivation, in addition to robust alterations across the whole brain functional connectome (De Havas et al., 2012; Kaufmann et al., 2015; Krause et al., 2017; Sämann et al., 2010; Tüshaus et al., 2017; Yeo et al., 2015). Finally during N3 sleep (also called deep, or slow wave, sleep), several studies reported a strong disruption of DMN connectivity and anti-correlation (Horovitz et al., 2009; Larson-Prior et al., 2011; Sämann et al., 2011), as well as a vanishing of FP connectivity (Spoormaker et al., 2012). Altogether, these results argue in favor of a progressive loss of functional segregation of brain networks from sleep onset to deep sleep, which might explain why the behavioral impairments at awakening are the most acute when individuals are awakened in N3 sleep. They also suggest that sleep inertia dissipation in the first half hour after awakening is associated with a progressive restoration of the brain networks' functional segregation.

This latter hypothesis has however never been experimentally tested. In order to fill in this gap, we designed a paradigm with simultaneous EEG-fMRI recordings to assess brain functioning during resting-state before a 45 min early-afternoon nap, 5 min after awakening and 25 min after awakening. This enabled us to investigate the dynamic of brain activity during the first half hour following awakening. Each resting-state scan was coupled with a mental calculation task (descending subtraction task, DST) in order to measure sleep inertia at the behavioral level (Tassi and Muzet, 2000). To our knowledge, this is the first neuroimaging study of sleep inertia using such a behavioral control. Furthermore, participants were partially sleep deprived on the night before and awakened, when possible in N3 sleep. As both sleep deprivation and N3 sleep have been associated with increased sleep inertia (Tassi and Muzet, 2000), our design allowed us to study sleep inertia in its most intensified form. This choice also had an ecological value since short nights compensated by a daytime nap are common in young adults (Faraut et al., 2017).

This paradigm provides a unique opportunity to study the brain and cognitive alterations during sleep inertia since it is the first one to provide both EEG-fMRI and behavioral data at both 5 and 25 min post-awakening. Using such a paradigm, we can observe the alterations that are specific to sleep inertia by contrasting behavioral and brain measures obtained at 5 min post-awakening to the ones obtained at pre-sleep. Likewise, the dissipation of sleep inertia can be evaluated by contrasting the brain and behavioral measures obtained at 5 min post-awakening to the ones obtained at 25 min post-awakening.

Our first hypothesis was that sleep inertia would be conspicuous both behaviorally and physiologically at 5 min post-awakening. Using a within-subject statistical design, we expected (1) a decrease in mental calculation performances at 5 min post-awakening compared to pre-sleep and 25 min post-awakening, reflecting the progressive dissipation of sleep inertia detrimental effects over the first half hour after awakening, (2) an intrusion of sleep-specific brain activity at 5 min post-awakening, i.e. a high spectral power of slow activity (EEG), a disrupted DMN functional connectivity, and a loss of brain networks' functional segregation (fMRI). Our second hypothesis was that the severity of these physiological and behavioral alterations would be positively correlated with sleep depth and duration. In other words, we expect that participants awakened in N3 sleep will have an intensified form of sleep inertia than participants awakened in N2 sleep and that this difference will be apparent on both behavioral and physiological measures. Finally, we expected correlations between connectivity measures, electrophysiological measures and behavioral measures of sleep inertia.

2. Methods

2.1. Participants

Fifty-five participants were included in the study (28 males, mean age ± standard deviation = 22.55 ± 2.41, range = 19–29). The subjects were informed of the study through an announcement sent to several mailing lists of Lyon University (Vallat et al., 2018). Participants were selected if they reported having a regular sleep-wake schedule, no difficulty falling asleep, being occasional or frequent nappers and having preferentially already done an MRI brain scan in the past few years. They had no history of neurological and psychiatric disorders and had no sleep disturbances (PSQI score = 4.60 ± 2.25). They provided written informed consent according to the Declaration of Helsinki and received monetary compensation for their participation. The study was approved by the local ethics committee (CCPPRB, Centre Leon Berard, Lyon, France).

2.2. Experimental design

The experimental design is presented in Fig. 1.

Evening and night. Participants arrived in the sleep unit of Le Vinatier Hospital (Lyon, France) at 8pm on the evening prior to the experimental day. From 8pm to 10pm, they underwent several personality and cognitive tests administered by R.V (results will be presented elsewhere). They were instructed to stay awake until 5am, after which they were allowed to sleep for 3 h until 8am in a bed of the sleep unit. The possible activities during the partial sleep deprivation were reading, making puzzles and watching movies. Energy drinks, caffeine, and physical activity were prohibited, and nurses regularly checked that the subjects did not fall asleep. As an additional control, participants wear a wrist actimeter (Actigraph link GT9x, Actigraph, FL, USA; sampling rate: 30 Hz) at the dominant hand in order to verify *a posteriori* that they did not fall asleep before 5am. In the morning, participants were offered breakfast and a shower and then occupied themselves (reading or internet) under the experimenters' supervision until the MRI session.

Day. After lunch at 11:30am, participants were conducted to the neuroimaging facility (CERMEP). During the first half hour, experimenters set up an MRI-compatible polysomnographic cap. Polysomnographic data were recorded continuously throughout the entire duration

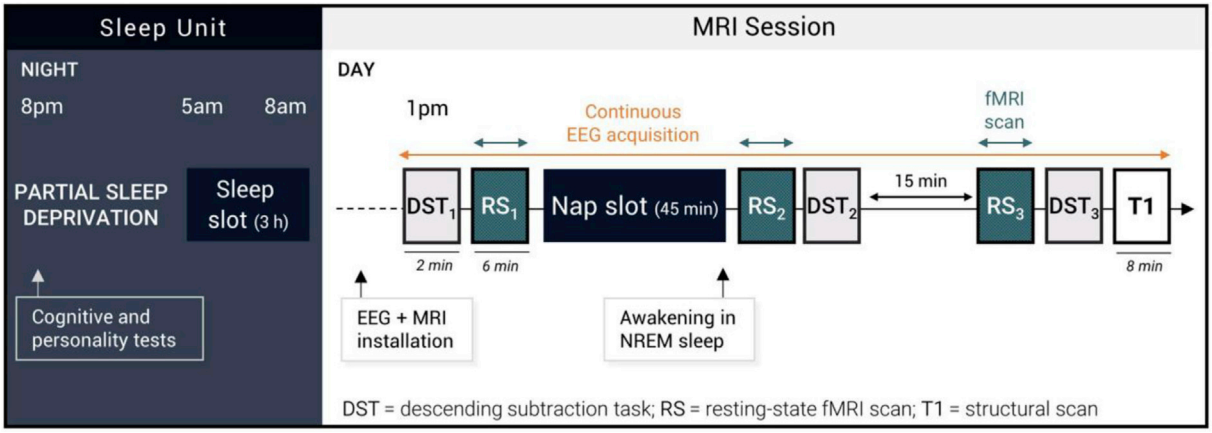

Fig. 1. | Experimental design.

of the subsequent MRI session (~2 h). Participants were then installed in the MRI scanner at about 1:20pm (1:17pm ± 13 min) and the eye-tracking camera was calibrated. To check the quality of the eye-tracking calibration participants read a 5 min cartoon in the scanner. Then, they did the mental calculation task for 2 min (see next section), just before the acquisition of the first resting-state fMRI scan. During the scan, the instructions were to remain awake and look at a fixation cross on the screen (the experimenters checked the eye-tracking camera during the whole scan to make sure that the participant did not close their eyes). At the end of the fMRI scan (1:39pm ± 14 min), participants were informed that they could rest or sleep during the next 45 min. We did not scan during the nap in order to facilitate sleep and because the primary goal of the study was to investigate the brain activity during wake just before and after awakening. Throughout the whole nap slot, the experimenters monitored the vigilance state of the participants in real-time by looking at the polysomnographic recording. After about 45 min (mean = 42 ± 5 min), participants were awakened by calling their first name and the second set of measurements (resting-state fMRI scan and mental calculation task) was done as quickly as possible after the awakening. After the second DST, subjects were asked to describe their sleep and dreams in the scanner and were also asked about the cartoon that they read just after the eye-tracker calibration (results will be reported elsewhere). About 25 min after awakening (23 ± 3 min), the third set of measurements (resting-state fMRI scan and mental calculation task) was performed and followed by an 8 min T1 anatomical scan. At the end of the experiment, participants completed a questionnaire about their thoughts during the 3 resting-state scans.

2.3. Behavioral task

To evaluate the effect of sleep inertia at a behavioral level, we used the DST, a mental calculation task which has been previously reported to be sensitive to sleep inertia (Dinges et al., 1985; Evans and Orne, 1975; Stampi et al., 1990). Subjects were presented with a three-digit number. They were instructed to subtract 9, saying the operation and the result aloud, and then continue by subtracting 8 from the remainder, then 7, and so on until they had to subtract 1. After this point, they were to start the cycle of descending subtractions again. They had to do the task for 2 min and were instructed to be as fast and accurate as possible. As this task has a substantial practice effect over the first trials (Dinges et al., 1985), participants were trained on the evening prior to the MRI session (they performed the task six times).

The outcome measures of the DST are (1) the total number of responses, which is an index of the speed of information processing, (2) the percentage of mistakes, which is an index of accuracy and (3) the percentage of correct responses, relative to pre-nap performances, which is an index of both speed and accuracy (Dinges et al., 1985). Since several studies reported that speed is generally more impaired than accuracy during sleep inertia (Trotti, 2017), we expected a significant decrement of post-nap performances especially for the total number of responses and percentage of correct responses. For each of these outcome measures, scores were entered as dependent variables into a mixed two-way repeated measures ANOVA with a between-subject group factor (two levels: N2 and N3 sleep, see results section) and a within-subject time factor (three levels: pre-sleep, 5 min post-awakening and 25 min post-awakening). In case of significance, post hoc tests were computed using two-sided T-tests corrected for multiple comparisons using the false discovery rate (FDR) and Hedges' g values were reported as a measure of effect size.

2.4. Polysomnography data collection and analysis

2.4.1. Actigraphy

The actimetric data were analyzed using the actigraph.sleepr R package.[1] Sleep scoring was initially performed using the Cole-Kripke algorithm (Cole et al., 1992). Subsequently, the Tudor-Locke algorithm (TudorLocke et al., 2014) was applied to compute the total sleep duration and wake after sleep onset (WASO), using the following settings: minimum sleep period length = 90 min, minimum inactive time to define bedtime = 5 min, minimum activity time to define waking time = 10 min.

2.4.2. Data collection

Polysomnography data were continuously recorded using a 15 channels MR-compatible cap designed for sleep studies, i.e. with a layout designed according to the American Academy of Sleep Medicine Guidelines (EasyCap, Brain Products GmbH, Gilching, Germany). It comprised 9 EEG electrodes placed according to the international standard 10/20 system (O1, O2, C3, C4, F3, F4, Fpz, M1, M2; with Cz as the reference and Afz as the ground), 2 EOG electrodes, 3 EMG electrodes, and an ECG electrode placed on the back of the participant. The sampling rate was 5000 Hz and an analog band-pass filter was set to 0.01–250 Hz. The helium pump was not disabled during data acquisition. The EEG amplifier (BrainAmp MR, Brain Products GmbH, Gilching, Germany) was positioned at the rear of the MRI scanner. The cable connecting the amplifier to the EEG cap was fixed in place using sandbags. The amplifier was connected to the PC interface in the control room via a fiber optic cable.

To allow for an online evaluation of the sleep stages during the fMRI session, a real-time pulse-artifact correction was applied using the BrainVision Recorder (version 1.2) and BrainVision RecView (version

[1] https://github.com/dipetkov/actigraph.sleepr.

1.4) softwares (Brain Products). This allowed to remove the cardioballistic artefact, which results from the pulse-related movement of the electrodes in the constant magnetic field of the scanner (importantly this artefact alters the EEG signal even outside of the scanning periods).

To ensure that the participants were keeping their eyes open during the resting-state scans, eye movements were monitored using an EyeLink 1000 fMRI eye-tracking system (SR Research, Ontario, Canada). Eye position was calibrated at the beginning of the experiment and monitored throughout. During all the resting-state scans, R.V. and P.R. checked in real-time that the participants were not closing their eyes using the online video and, when appropriate, reminded the subjects to keep their eyes open through a microphone.

2.4.3. Data preprocessing

To allow further analyses, data were first segmented, for each subject, into four subsets: the nap (~45 min) and the first, second and last resting-state scans (6 min each). Artifacts related to gradient switching and cardiac pulse (cardioballistic effect) were removed in each of these segments using semi-automatic MRI artifact rejection algorithms (Allen et al., 2000, 1998) implemented in Brain Vision Analyzer 2.1 software (Brain Products). Polysomnographic data were then downsampled to 500 Hz and bandpass filtered between 0.1 and 40 Hz.

2.4.4. Sleep staging

Offline sleep staging was performed using epochs of 30 s visualized in an open-source in-house software (Combrisson et al., 2017). Sleep staging was done visually by an expert rater (R.V.; Combrisson et al., 2017; Vallat et al., 2018, 2017). Sleep staging was performed according to the standard guidelines by the American Association of Sleep Medicine (Iber, 2007; Silber et al., 2007). The amplitude of the frontal derivations was used to determine N3 sleep. Fig S1 shows the hypnogram and a 20 s EEG segment during the nap slot for one subject from the N3 group. The following sleep statistics were extracted from the hypnogram of each subject and then averaged across subjects: duration of wake, N1 sleep, N2 sleep, N3 sleep and REM sleep (min), longest period of uninterrupted N2 sleep and N3 sleep (min), total sleep time (min, computed as the sum of N1, N2, N3 and REM sleep duration), total dark time (min, duration from the beginning to the end of the nap slot), sleep efficiency (%, computed as the ratio between the total sleep time and the total dark time), as well as the latencies between the awakening and the start of the first and second post-awakening resting-state scans (min).

2.4.5. Spectral analyses

Spectral analyses were conducted on the segmented EEG data corresponding to the first, second and third resting-state fMRI scans (6 min each). Power spectral density (PSD) were estimated for four frequency bands (delta = 0.5–4.5 Hz, theta = 4.5–7.5 Hz, alpha = 7.5–11.5 Hz, beta = 11.5–30 Hz) using the *psd_welch*[2] function implemented in the MNE-python package (Gramfort et al., 2014) with a 4-s sliding window and a 1-s step. PSD were computed for 6 channels (F3, F4, C3, C4, O1, O2) and then averaged to obtain a single value per frequency band per resting-state scan per subject. Three EEG channels (Fpz, M1, M2) were not included in the spectral analyses because of an overall bad data quality, mainly resulting from the MR environment. Before applying further statistical analyses, PSD values were transformed to geometric standard scores, which are more appropriate than arithmetic standard scores when the raw scores follow a log-normal distribution (Kirkwood, 1979), using the following equation:

$$z(x) = ln(x / \mu_g) / ln(\sigma_g)$$

Where x, μ_g and σ_g are the raw PSD scores, geometric mean and geometric standard deviation, respectively.

Then, for each frequency band, PSD values were entered in a mixed two-way repeated measures ANOVA with a between-subject group factor (two levels: N2 sleep and N3 sleep) and a within-subject time factor (three levels: pre-sleep, 5 min post-awakening and 25 min post-awakening). In case of significance, post hoc tests were computed using FDR-corrected two-sided T-tests.

2.5. MRI data collection

MRI scans were obtained from a MAGNETOM Prisma 3T scanner (Siemens Healthcare, Erlangen, Germany) at the Primage neuroimaging facility (CERMEP, Lyon, France). Structural MRI were acquired with a T1-weighted (0.9 mm isotropic resolution) MPRAGE sequence and functional MRI data with a T2*-weighted 2D gradient echo planar imaging sequence (EPI) with 180 vol (TR/TE = 2000/25 ms, flip angle = 80°, voxel size = 2.68 × 2.68 × 3 mm, slices = 40, duration = 6 min). Functional and anatomical scans were performed using a 20-channel head coil. The coil was foam-padded to improve subject comfort and restrict head motion.

Importantly, scan length is an important factor in resting-state fMRI, with longer scan times typically increasing the reproducibility of within-subjects functional connectivity estimates (Anderson et al., 2011). Because of the inherently time-constrained nature of our paradigm, we chose a 6 min scan duration which provides *"a reasonable trade-off between time/robustness of resting-state networks functional connectivity"* (Soares et al., 2016). Indeed, several studies have shown that correlation values from functional connectivity matrices within and between brain networks stabilize within 4–5 min of data (Van Dijk et al., 2010; Whitlow et al., 2011).

2.6. fMRI analysis

Preprocessing and quality check was performed using the CONN toolbox[3] version 17f (Whitfield-Gabrieli and Nieto-Castanon, 2012). Preprocessing was done according to the standard pipeline implemented in the CONN toolbox and included functional realignment, slice-timing correction, coregistration to structural scan, spatial normalization and spatial smoothing using a 6-mm full-width at half-maximum isotropic Gaussian kernel filter. Individual T1 images were segmented into gray matter, white matter and cerebrospinal fluid tissue maps. Functional and structural images were then normalized to the MNI152 space (Montreal Neurological Institute). Functional images underwent artifact and motion regression in the Artifact Detection Toolbox[4] using the following criteria to define outliers: global signal intensity changes greater than 9 standard deviations and movement exceeding 2 mm. SPM motion parameters and outliers were included as covariates in further connectivity analyses.

Six resting-state functional brain networks and their main regions of interests (ROIs) were defined from a brain parcellation atlas natively implemented in the CONN toolbox. This atlas was obtained using an independent component analysis on 467 subjects from the Human Connectome Project (Smith et al., 2013). In addition to these six cortical networks, a set of subcortical areas (hippocampus, thalamus, and amygdala) was defined from the Harvard-Oxford maximum likelihood subcortical atlas. The full list and spatial map of networks and their corresponding regions can be found in Table S1 and Fig. S2, respectively.

Functional connectivity analyses were achieved using the CONN toolbox version 17f. First, we performed a denoising step including a regression of the 6 motion correction parameters and their corresponding first-order temporal derivatives, as well as a regression of the principal components derived from anatomical noise ROIs (i.e. the white matter

[2] https://martinos.org/mne/stable/generated/mne.time_frequency.psd_welch.html.

[3] http://www.conn-toolbox.org/.

[4] https://www.nitrc.org/projects/artifact_detect/.

and cerebrospinal fluid, which signals are unlikely to be related to neural activity) in order to remove physiological confounds (Behzadi et al., 2007). The resulting BOLD time series were band-pass filtered (0.008 - 0.09 Hz) to further reduce noise and increase sensitivity (Weissenbacher et al., 2009). Then, functional connectivity matrices were calculated for each subject by extracting the mean BOLD time series of each ROI of a given network and by correlating them with the average BOLD time series of every other ROI from this network or the other networks included in the analysis. Because there were 32 ROIs included in the analysis, the resulting skew-symmetric functional connectivity matrices comprised 496 ROI-to-ROI pairwise correlation coefficients (i.e. n*(n-1)/2). The correlation coefficients were then standardized using the Fisher Z-transformation:

$$z := \frac{1}{2} \times ln\left(\frac{1 + r}{1 - r}\right)$$

Second-level statistical comparisons were performed for N3 and N2 group separately. We used two-sided pairwise T-tests with an FDR-corrected (seed-level) alpha threshold set a 0.05. For each group, three contrasts were performed: pre-nap versus 5 min post-awakening, pre-nap versus 25 min post-awakening, and 5 min versus 25 min post-awakening.

Second, to make these results more meaningful with regards to the global organization of brain networks, we then extracted the average functional connectivity within and between brain networks. These values were obtained by computing the arithmetic mean of all pairwise correlation coefficients. The resulting averaged connectivity values were subsequently entered into a mixed two-way repeated measures ANOVA with a between-subject group factor (two levels: N2 sleep and N3 sleep) and a within-subject time factor (three levels: pre-sleep, 5 min post-awakening and 25 min post-awakening). In case of significance, post hoc tests were computed using FDR-corrected two-sided T-tests.

2.7. Correlations

Finally, we computed the correlation coefficients between several dependent variables, including the performances at the DST, the EEG spectral measures, the sleep parameters and the average functional connectivity within and between brain networks. Correlation coefficients were computed using two-sided Pearson product-moment correlation coefficient (p-uncorrected $< .05$). Because of the huge number of possible correlations, we restrained our analysis to specific a priori hypotheses, which are detailed in the results section.

2.8. Statistics

The mixed two-way repeated measures ANOVA were computed using the *ezANOVA*[5] package implemented in R. In case of significance, post hoc tests were computed using two-sided T-tests FDR-corrected for multiple comparisons using the Benjamini–Hochberg procedure. They were performed using the R package *lsmeans*.[6] For each post hoc comparisons, we also report the Hedges' g as a measure of effect size. Effect sizes were computed using the R package *effsize*.[7] Correlations were computed using the *pearsonr*[8] function implemented in the SciPy python package. On all the following graphs, error bars represent 95% bootstrapped confidence intervals (2000 permutations) computed using the *seaborn*[9] python package.

[5] https://github.com/mike-lawrence/ez.

[6] https://cran.r-project.org/web/packages/lsmeans/vignettes/using-lsmeans.pdf.

[7] https://github.com/mtorchiano/effsize.

[8] https://docs.scipy.org/doc/scipy/reference/generated/scipy.stats.pearsonr.html.

[9] https://github.com/mwaskom/seaborn.

3. Results

3.1. Sleep parameters

3.1.1. Night

Actigraphy results showed that the participants slept an average of 152.67 ± 15.32 min (range = 95–194 min) on the night before the MRI session. No participant fell asleep during the sleep deprivation period (i.e. from 10pm to 5am). The average WASO was 13.35 ± 9.16 min (range = 1–51 min). However, it should be noted that a previous study comparing wrist actigraphy with polysomnography reported that the former method overestimates WASO (Slater et al., 2015).

3.1.2. Nap

Despite the sleep deprivation, 20 out of 55 participants were not able to reach or maintain NREM sleep deeper than N1 during the 45-min nap slot in the scanner. This result was not a surprise given the discomfort and stress inherent to the MR environment (Duyn, 2012). These 20 subjects were not included in further analyses. One subject out of the 35 remaining was discarded because of a technical failure during data acquisition, leading thus to a total of 34 participants in the final analysis (15 males, mean age = 22.67 ± 2.48, range = 19–29).

We further divided these participants as a function of the sleep stage in which they were prior to awakening. Twenty participants were awakened in N3 sleep (N3 group) and 14 participants were awakened in N2 sleep (N2 group). This allowed us to conduct fine-grained comparisons of the behavioral and brain alterations following awakening from N2 and N3 sleep. Note that we did not include participants who only reached N1 sleep since awakening from N1 sleep is known to induce little to no sleep inertia (Tassi and Muzet, 2000) and was therefore of little interest with regards to our initial goal. Furthermore, in the N1 group, there was a lot of variability and heterogeneity in the sleep structure of the participants as well as in the vigilance state at the time of awakening.

Means of the main sleep parameters in the N2 and N3 groups are presented in Table 1. Importantly, there was no group difference in the delay between the awakening, on one hand, and, the first and second post-awakening resting-state fMRI scan on the other hand.

3.2. Behavioral performances

Behavioral performances at the DST are presented in Fig. 2.

3.2.1. Total number of responses

As expected, we observed a significant main effect of time in the total number of responses, $F\ (2,64) = 4.0$, $p = .02$. The total number of responses was lower at 5 min post-awakening as compared to 25 min post-awakening (p-fdr = .04, Hedges' g = .19). There was a tendency for a reduced total number of responses at 5 min compared to pre-nap (p-fdr = .07, g = .13). No group effect or interaction was found for the total number of responses.

3.2.2. Percentage of mistakes

This decrease in calculation speed at 5 min post-awakening was not associated with an increase in the percentage of mistakes. However, there was a significant main effect of group ($F\ (2,64) = 5.8$, $p = .02$), as well as a significant interaction between time and group, $F\ (2,64) = 3.6$, $p = .03$. Post hoc tests revealed that the N2 group had a higher percentage of mistakes than the N3 group (p-fdr = .002, g = .64), and specifically at 5 min post-awakening (p-fdr = .02, g = .99). This effect should nevertheless be interpreted with caution since it was mainly driven by one participant of the N2 group who made 35% of mistakes in the second run. The interaction was not significant anymore when this subject was removed from the analysis, $F\ (2,62) = 2.1$, $p = .13$.

Table 1
| **Sleep parameters (mean ± SD) of the subjects in the N3 (n = 20) and N2 (n = 14) groups.** TST = total sleep time, SE = sleep efficiency (%), Wake (W), N1, N2, N3, REM = total duration of each sleep stage in minutes. Long-N2 = longest period (min) of uninterrupted N2 sleep. Long-N3 = longest period of uninterrupted N3-sleep. LAS1 = latency (min) between the awakening and the start of the first post-awakening resting-state fMRI scan. LAS2 = latency (min) between the awakening and the start of the second post-awakening resting-state fMRI scan.

Group	TST	SE	W	N1	N2	N3	REM	Long-N2	Long-N3	LAS1	LAS2
N2	38.2 ± 7.1	87.4 ± 9.6	9.1 ± 4.0	14.2 ± 7.6	21.6 ± 6.2	2.9 ± 4.4	0	11.3 ± 5.2	2.7 ± 4.2	3.7 ± 1.9	23.8 ± 3.7
N3	37.3 ± 5.0	87.5 ± 7.4	9.2 ± 4.2	9.0 ± 5.7	17.7 ± 4.9	11.0 ± 4.6	0	14.4 ± 3.9	10.3 ± 4.2	4.8 ± 3.9	24.4 ± 4.2
T-test	.68	.99	.95	.03	.05	<.001	–	.06	<.001	.34	.63

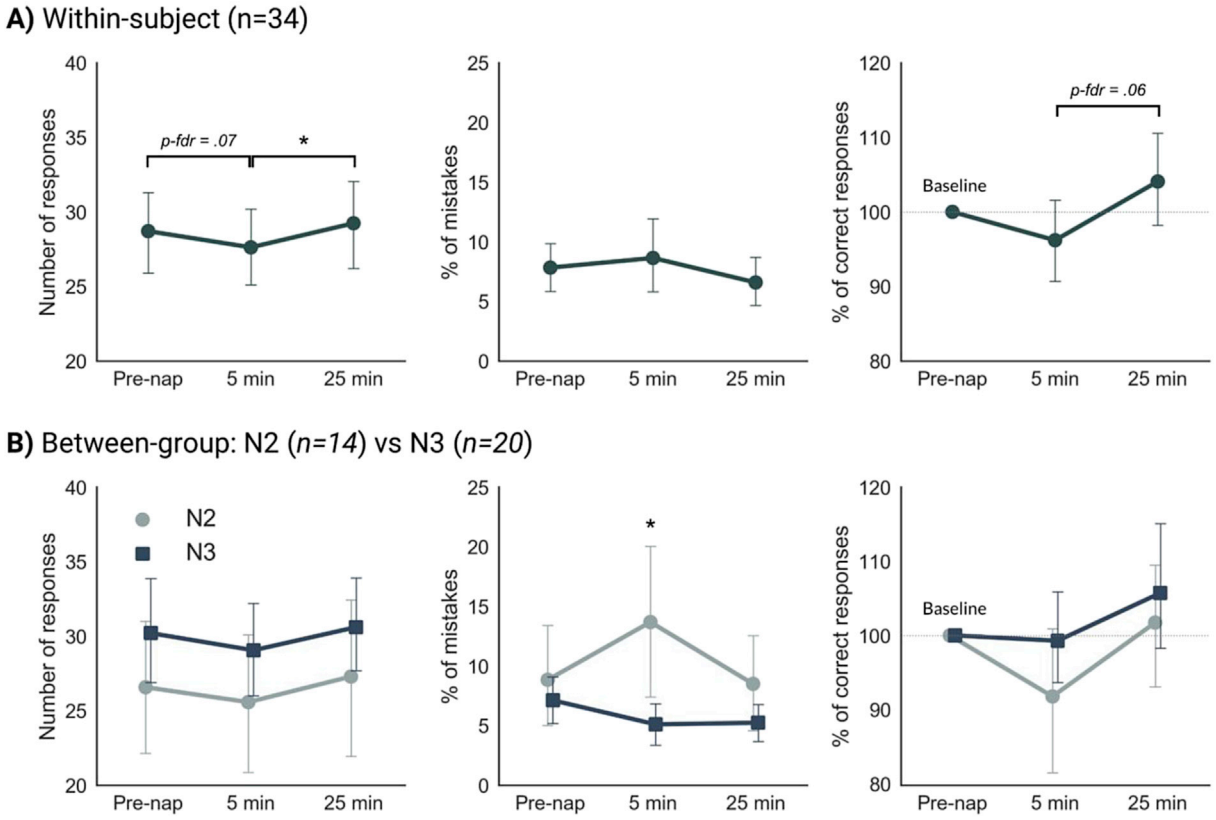

Fig. 2. | **Behavioral performances at the descending subtraction task (DST)** (A) Within subject comparisons (i.e. main effect of time). for the total number of responses (left), percentage of mistakes (middle) and percentage of correct responses relative to pre-nap performances (left). (B) Between group comparisons (N2 vs N3). Dashed horizontal lines in the left panel indicate the 100% baseline pre-nap performances. Error bars represent bootstrapped 95% confidence intervals. *p < .05, **p < .01. All p-values are corrected for multiple comparisons using the false discovery rate (FDR).

Percentage of correct responses

We also found a significant main effect of time for the percentage of correct responses, F (2,64) = 3.13, p = .050. Post hoc tests revealed a tendency for a lower percentage of correct responses at 5 min post-awakening compared to 25 min post-awakening (p-fdr = .06, g = .44). No group effect or interaction was found.

3.3. EEG power spectral density

Four out of the 34 subjects were not included in the PSD statistical analyses because of a technical failure during polysomnographic data acquisition (n = 1) or bad data quality (n = 3). These outliers were defined using the following rule: grand averaged PSD above or below 2 standard deviations in two or more frequency bands. An additional outlier rejection was applied for the 30 remaining participants by removing observations below the 1st and above the 99th percentiles. This latter outlier rejection did not result in any subjects being removed from the analyses.

PSD results are presented in Fig. 3. As expected, we found a main effect of time for the delta frequency band, F (2,56) = 3.48, p = .038. There was an almost significant tendency for increased delta power at 5 min post-awakening compared to both pre-nap (p-fdr = .051, g = .32) and 25 min post-awakening (p-fdr = .051, g = .29). Second, there was also a main effect of time for the alpha frequency band, F (2,56) = 8.75, p = .001. Alpha power was significantly increased at 25 min post-awakening compared to both pre-nap (p-fdr = .0009, g = .57) and 5 min post-awakening (p-fdr = .050, g = .29). Third, we observed a main effect of time for the beta frequency band, F (2,56) = 5.38, p = .007. Beta power was significantly increased at 25 min post-awakening compared to both pre-nap (p-fdr = .03, g = .37) and 5 min post-awakening (p-fdr = .03, g = .42). Fourth, we found a main effect of time for the delta-beta ratio, F (2,56) = 4.73, p = .013. Post hoc tests revealed that the delta-beta ratio was significantly increased at 5 min post-awakening compared to pre-nap (p-fdr = .03, g = .52). There was no main effect of time for the theta frequency band, and neither was there a significant effect of group or interaction for any of these frequency bands. The group

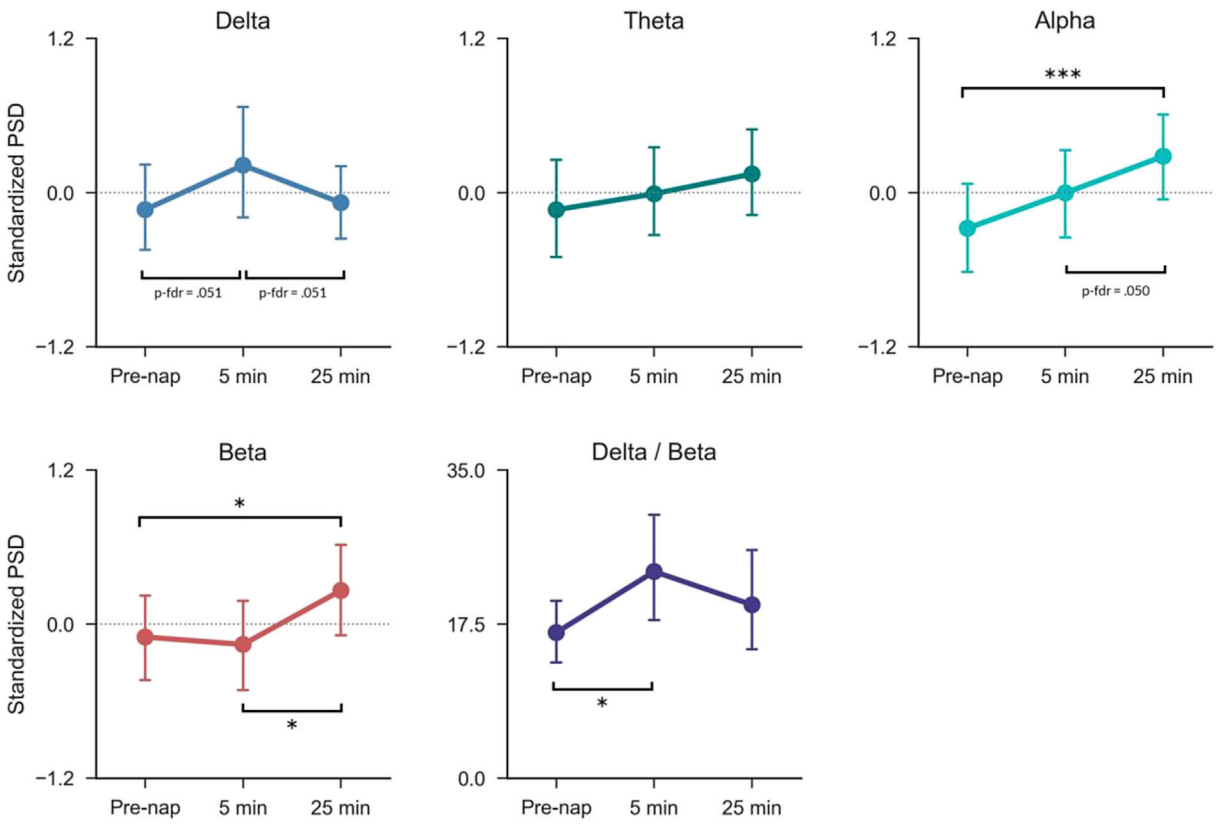

Fig. 3. | **EEG spectral power during the three resting-state fMRI scans.** As no significant main effect of group or interaction was found, only the average PSD values of the two groups are depicted in this figure. Error bars represent bootstrapped 95% confidence intervals. *p < .05. ***p < .001. All p-values are corrected for multiple comparisons using the false discovery rate (FDR).

means are reported in Fig S3.

3.4. Brain networks functional connectivity

3.4.1. ROI-to-ROI analysis

ROI-to-ROI results are presented in Fig. 4.

As expected, awakening from N3 sleep was associated with strong disruptions in functional connectivity. ROI-to-ROI analyses demonstrated a strongly disrupted pattern of functional connectivity between several brain networks at 5 min post-awakening compared to both pre-nap and 25 min post-awakening (Fig. 4A). Perhaps the most important finding was an increased functional connectivity at 5 min post-awakening compared to pre-nap between several regions of the DMN and the DAN. This pattern was also observed, albeit to a lesser extent, when contrasting the 25 min and 5 min post-awakening scans. Finally, the last contrast (pre-nap > 25 min) revealed some differences in the functional connectivity at 25 min and at pre-sleep, namely between the superior region of the SM network on one side and regions of the salience and visual networks on the other side (Fig S4).

Interestingly, awakening from N2 sleep induced a different pattern of disruption (Fig. 4B). Compared to pre-nap, the functional connectivity at 5 min post-awakening was increased between the hippocampus and regions of the DMN, and between regions of the SM and those of the salience network. Contrary to N3 sleep, we also observed some significant decreases in pairwise functional connectivity, namely between regions of the SM and those of the FP networks, as well as between the hippocampus and the regions of the SM networks. For the N2 group, however, the most visible changes were observed when contrasting the 25 min and 5 min post-awakening scans. As compared to 25 min post-awakening, the functional connectivity at 5 min post-awakening was significantly increased between several regions of the visual and salience network, and significantly decreased between several regions of the SM and FP networks. As for the N3 group, we did observe some changes in the pre-nap versus 25 min post-awakening contrast. Namely, the functional connectivity was decreased at 25 min post-awakening compared to pre-nap in several regions of the salience, DMN, visual and FP networks (Fig S4).

3.4.2. Average within and between network functional connectivity

An easier way to understand these results is to look at the average functional connectivity within and between brain networks. Doing so, we were able to confirm and extend the ROI-to-ROI results. First, there was no significant effect of time, or group, or any interaction between time and group when we only considered the within-network average functional connectivity (i.e. connectivity within a specific brain-network, Fig. 5, *top*). However, we did find several significant effects in the pairwise between network functional connectivity (i.e. the average functional connectivity between two networks, Fig. 5, *bottom*). Consistent with the above results, a two-way repeated measures ANOVA revealed a significant main effect of time in the functional connectivity between the DMN and the DAN (F (2,64) = 4.0, p = .02), as well as a significant interaction between group and time, F (2,64) = 3.7, p = .03. Post hoc tests revealed a significantly increased connectivity between these networks at 5 min post-awakening compared to pre-nap (p-fdr = .03, g = .53), as well as a tendency for a significant increase at 5 min compared to 25 min post-awakening (p-fdr = .08, g = .39). Consistent with the ROI results, post hoc tests showed that the interaction effect at 5 min post-awakening was mainly driven by the N3 group (p-fdr = .02, g = 0.89).

Second, we also found a significant main effect of time in the functional connectivity between the DMN and salience network, F (2,64) = 4.1, p = .02. Post hoc tests revealed a significantly increased connectivity between these networks at 5 min post-awakening compared to pre-nap (p-fdr = .03, g = .55), as well as a tendency for a significant increase at 25 min post-awakening compared to pre-nap (p-fdr = .08, g =

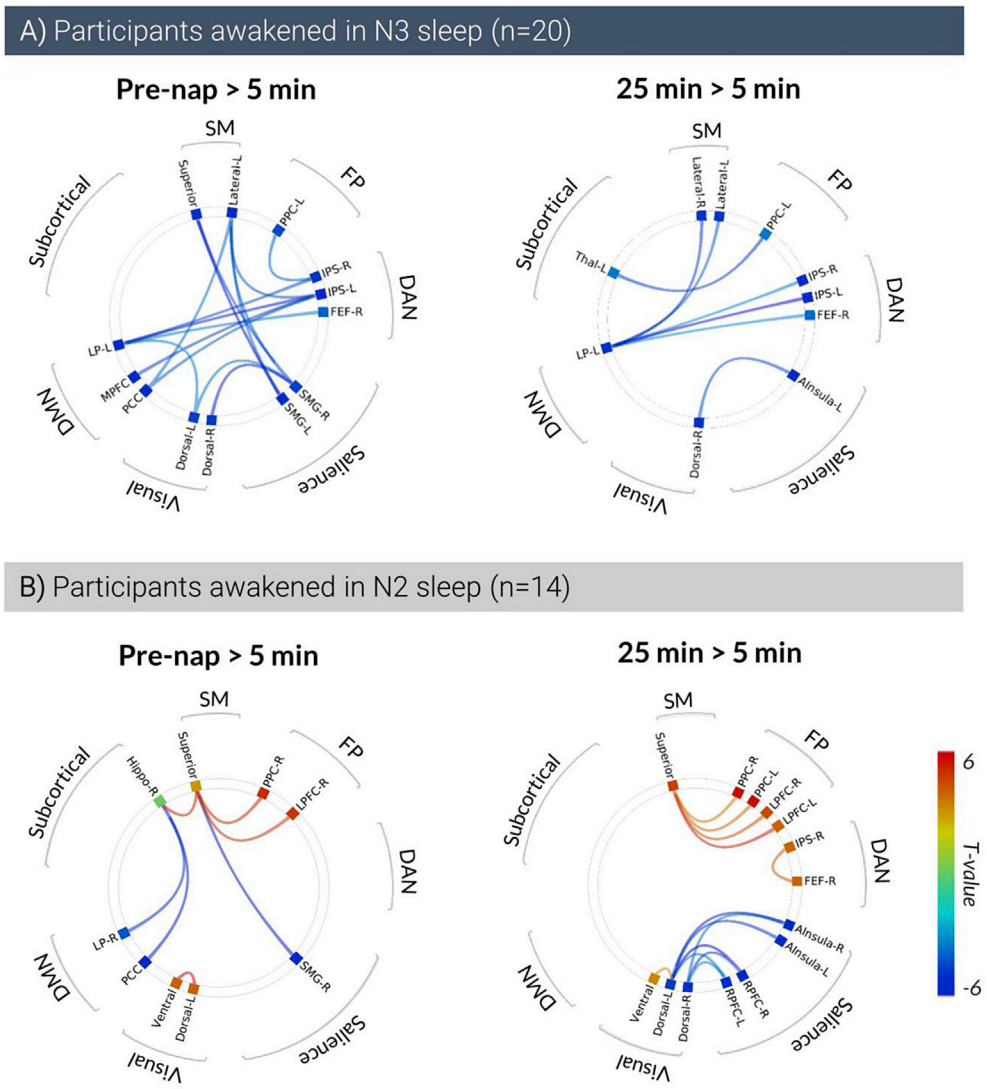

Fig. 4. | **Functional connectivity results.** ROI-to-ROI results for the two contrasts (*left*, pre-nap versus 5 min post-awakening; *right*, 25 min versus 5 min post-awakening) and the two groups (*top*, N3 group, *bottom*, N2 group). Blue connections indicate regions with significantly increased pairwise connectivity (two-sided paired t-tests, p-fdr < .05) at 5 min post-awakening. Orange-red connections indicate regions with significantly decreased pairwise connectivity at 5 min post-awakening.

.38). In addition, there was also a main effect of group (F (2,64) = 5.6, p = .02), indicating that participants who were awakened in N3 sleep had an overall higher functional connectivity between these two networks than those who were awakened in N2 sleep (p-fdr = .003, g = .59).

Finally, we also observed a significant interaction between group and time in the functional connectivity between the DMN and SM network, F (2,64) = 5.3, p = .008. Post hoc tests showed a significant increase in the functional connectivity between these two networks at 5 min post-awakening in the N3 group as compared to the N2 group (p-fdr = .004, g = .99).

3.4.3. *Correlations between spectral, fMRI and behavioral measures*

The first hypothesis that we wanted to test was to see whether the functional connectivity and/or EEG spectral power during the pre-nap resting-state scan could be predictive of the amount of NREM sleep (excluding N1 sleep) during the nap. To this end, we computed the correlation coefficients between the cumulative duration of N2 and N3 sleep (i.e. sum of N2 sleep duration and N3 sleep duration, referred to hereafter as the duration of N2/N3 sleep) on the one hand and the pre-nap PSD and average within- and between-networks functional connectivity values on the other hand. The only significant finding was a negative correlation between the duration of N2/N3 sleep and the DMN average functional connectivity (r = −0.4, n = 34, p-unc = .018; Fig. 6, *left*). In other words, the subjects who slept the most were also the ones with the lowest DMN functional connectivity right before the nap. Interestingly, we also found a tendency for a negative correlation between the duration of N2/N3 sleep and the average connectivity before the nap between the DMN and DAN (r = −0.31, n = 34, p-unc = .08), and between the DMN and the FP network (r = −0.31, n = 34, p-unc = .07).

Second, we tested whether the duration of N2 and N3 sleep during the nap could be predictive of the functional connectivity and/or EEG spectral power during the 5 min post-awakening resting-state scan. To this end, we computed the correlation coefficients between the duration of N2/N3 sleep on the one hand and the 5 min post-awakening PSD and average within- and between-networks functional connectivity values on the other hand. Here again, the only significant finding was a negative correlation between the duration of N2/N3 sleep and the DMN average functional connectivity (r = −0.41, n = 34, p-unc = .015, Fig. 6, *middle*). Consistent with the above, this suggests that disrupted DMN functional connectivity could be a marker of sleepiness, which applies both before sleep when one is tired but also immediately after sleep when one is in the sleep inertia state. In line with this, we found that the average DMN functional connectivity at 25 min post-awakening was not correlated with the duration of N2/N3 sleep during the nap (r = 0.02, n = 34, p-unc = .91, Fig. 6, *right*).

Altogether, these results suggest that the functional connectivity within the DMN, and between the DMN and other task-positive networks could be predictive of subsequent sleep duration. Note that a second

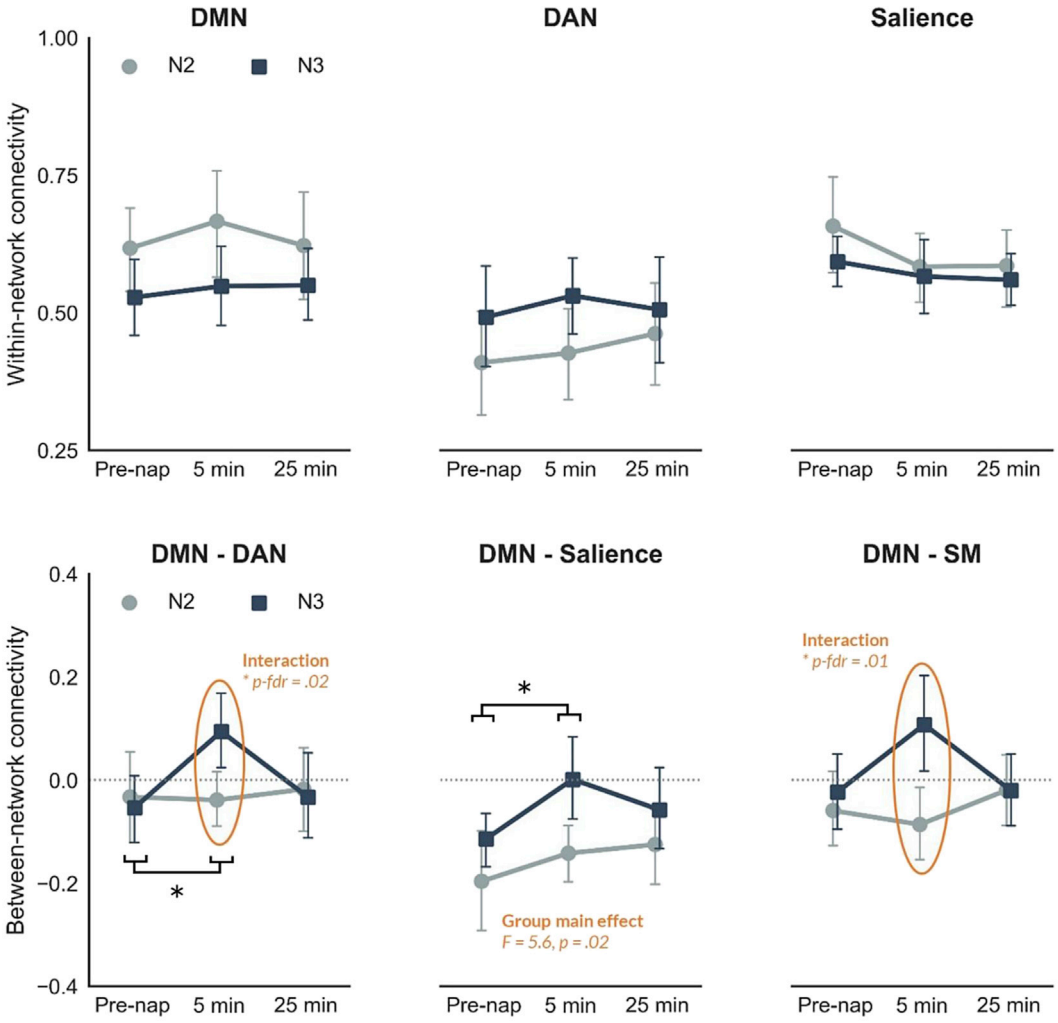

Fig. 5. | **Average within and between network functional connectivity.** *Top.* Average within-network pairwise functional connectivity. We did not find any significant main effect of time, group, or interaction between group and time for the average functional connectivity within each of the 7 networks considered in the analysis (only 3 are depicted). *Bottom.* Average between-network pairwise functional connectivity. Black lines illustrate post hoc comparisons in case of a significant main effect of time. Orange ellipses highlight significant post hoc comparisons in case of a significant interaction between time and group factors. Error bars represent bootstrapped 95% confidence intervals. *p < .05. All p-values are corrected for multiple comparisons using the false discovery rate.

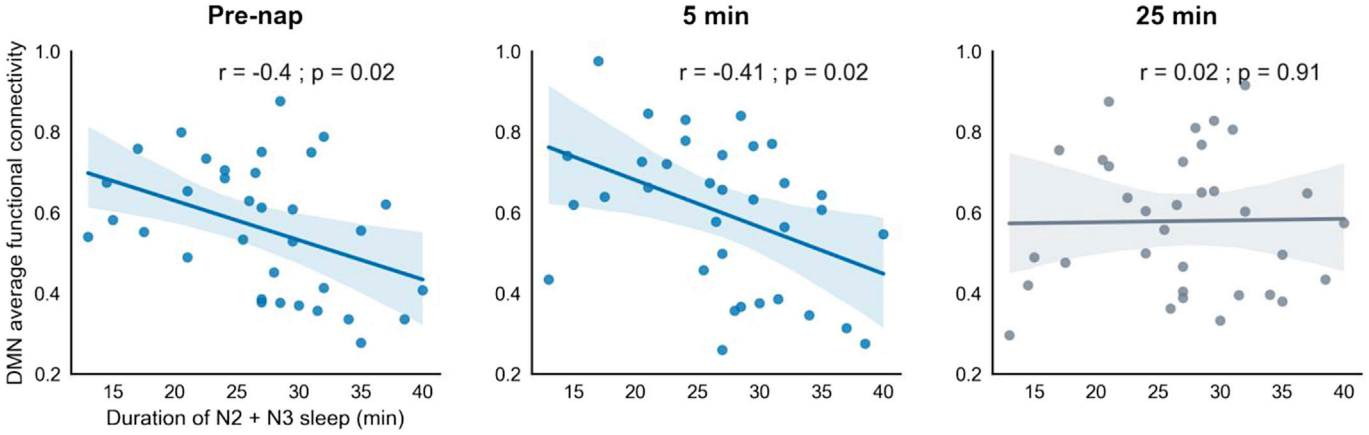

Fig. 6. | Correlations between sleep duration (N2 + N3 sleep, excluding N1 sleep) and average DMN functional connectivity. Blue colors indicate significant correlations. Error bars represent bootstrapped 95% confidence intervals.

possible explanation for the correlation between the duration of N2/N3 and the DMN functional connectivity at pre-nap and 5 min could be that DMN functional connectivity is correlated across the scans. In line with this, we have found that DMN functional connectivity is consistently correlated across scans (Fig S5), thus supporting the idea of a trait component of DMN functional connectivity (Mueller et al., 2013; Finn

et al., 2015). This does not explain, however, the absence of correlation between the duration of N2/N3 and the DMN functional connectivity at 25 min post-awakening, which could rather be explained by the dissipation of sleep inertia.

Third, we tested whether the average functional connectivity within and between brain networks could be related to the EEG. To this aim, we computed the correlation coefficients between the PSD in every frequency bands and every within and between networks average functional connectivity values, extracted from each of the three resting state scans. The findings support our previous hypotheses and results showing that sleep inertia is characterized by increased EEG delta power and reduced anti-correlation between task-positive and task-negative networks. Indeed, we found a positive correlation between the EEG delta power on the one hand and, on the other hand, the average functional connectivity between the DMN and the DAN ($r = 0.40$, $n = 30$, p-unc = .03, p-FDR = .44; Fig S6).

Fourth, we tested whether the behavioral performances at the DST could be related to physiological measures (i.e. brain networks functional connectivity and EEG spectral power). To do this, we computed the correlation coefficients between the outcome measures of the DST and the physiological measures, at each of the three measurement points. Strikingly, we found that the percentage of mistakes was positively correlated with the average connectivity in the DMN ($r = 0.38$, $n = 34$, p-unc = .0025, p-FDR = .58; Fig S6). This fits well with the well-known result that activation of the DMN during a task is associated with worsened performance and increased lapses of attention (Weissman et al., 2006). Neither this correlation nor the previous one (delta and DMN-DAN) did survive FDR correction for multiple comparisons but as they were expected an uncorrected threshold can be considered. In addition, one should keep in mind that the DST was performed before or after the resting-state scans and not during them, which may have diminished the strength of the correlation between the 2 measures.

4. Discussion

In a recent review on sleep inertia, Trotti (2017) proposed as first item on a 5-items research agenda that *"if as suggested by PET imaging, resumption of normal waking cognition on awakening requires reorganization of cognitive networks, can other functional neuroimaging and/or neurophysiologic studies better delineate these necessary network changes?"*. The present study aimed at addressing this latter issue by characterizing the behavioral performance, EEG spectral power, and brain network functional connectivity at awakening of an early-afternoon nap. We investigated this issue at awakening of both N2 and N3 sleep, which allowed us to compare the behavioral and physiological characteristics of the waking state immediately following awakenings from these two sleep stages.

4.1. Impaired mental calculation performance at awakening

As expected and regardless of the sleep stage preceding awakening, we found significant decrement in the total number of responses at awakening as compared to 25 min post-awakening, as well as a tendency for a reduced total number of responses at awakening compared to pre-nap (Fig. 2A). These results confirm that the detrimental effects of sleep inertia are maximum at awakening and that they progressively dissipate across the first half hour following awakening. The absence of significant difference in the percentage of mistakes is consistent with the generally held view that speed is more impaired than accuracy at awakening (Tassi and Muzet, 2000; Trotti, 2017).

The relatively smaller decrement in performances observed in the present study compared to previous studies that have used the DST immediately after awakening (Dinges et al., 1985; Evans and Orne, 1975; Stampi et al., 1990) may be accounted for by the delay between the awakening and the task, which was about 10 min in our study (i.e. duration of the resting-state fMRI scan = 6 min plus the average delay between awakening and the scan onset = 4 ± 2 min, see Table 1). We indeed chose to favor the estimation of functional connectivity over the estimation of behavioral performances at awakening. For this reason, the behavioral effects measured in our study most likely underestimate the behavioral impairment at awakening.

4.2. Modified EEG characteristics at awakening

Sleep inertia was also apparent in the EEG spectral power. In agreement with previous results, we found that EEG delta power was increased at 5 min post-awakening compared to pre-nap and 25 min post-awakening. The delta/beta ratio was also increased at 5 min post-awakening compared to pre-nap. These findings support the hypothesis that an increased delta activity in the first minutes after awakening may represent the EEG signature of sleep inertia (Ferrara et al., 2006; Gorgoni et al., 2015; Marzano et al., 2011).

Interestingly, we also found an increased EEG power in the alpha and beta frequency bands at 25 min post-awakening compared to measures at the two other time points (Fig. 3). According to the various functional role attributed to an increased power in the alpha band (i.e. signature of an increased vigilance: Davidson et al., 2000, Cantero et al., 2002, McKinney et al., 2011; signature of decreased vigilance: Pfurtscheller et al., 1996; Benca et al., 1999; Drapeau and Carrier, 2004), our results could indicate an increase or a decrease of vigilance in the last resting-state scan. However, the increase in beta power may rather indicate an increased arousal during the last resting-state scan (Davidson et al., 2000), when the fatigue caused by both the partial sleep deprivation and the sleep inertia were dissipated. Coherently with this hypothesis, although not statistically significant, the percentage of correct responses at 25 min post-awakening was slightly higher than before sleep (104%). Since sleep inertia has been previously reported to last between 20 and 30 min (Tassi and Muzet, 2000), one may conclude that our last resting-state scan was at the crossroads of the complete dissipation of the negative effect of sleep inertia and the beginning of the well-documented positive - and arousing - effect of napping (Faraut et al., 2017).

4.3. Disruption of brain functional connectivity at awakening

The functional connectivity between brain networks was strongly disrupted at 5 min after awakening from sleep compared to both pre-sleep and 25 min post-awakening (Fig. 5). Consistent with our hypotheses, we observed a decrease of the anti-correlation between regions of networks that are normally (i.e. during stabilized wakefulness) anti-correlated, namely between task-negative (DMN), and task-positive (DAN, salience, SM) networks. Such a loss of functional *segregation* between networks has been reported during N2 and N3 sleep (Picchioni et al., 2013) as well as during wake after a full night of sleep deprivation (Krause et al., 2017).

Our results therefore support the idea that sleep inertia is associated with an intrusion of sleep-specific features into wake brain activity, and shows that it is not only an intrusion of sleep-specific EEG activity but also an intrusion of sleep-specific functional connectivity (i.e. a reduced anti-correlation between the DMN and task-positive networks, as predicted by Balkin et al., 2002). In addition, the expected positive significant correlation between the EEG delta power and the functional connectivity between the DMN and the DAN brings further evidence of this tripartite relationship between increased slow EEG activity, reduced anti-correlation between brain networks, and sleep inertia (Fig S6).

These findings are well in line with a recent study reporting that caffeine ingestion, probably one of the most widespread practice to counteract the detrimental effect of sleep inertia, significantly enhances *"the anti-correlation between the default mode network and task-positive networks."* (Wong et al., 2013). As such, our results provide further support for the adenosine-based theory of sleep inertia, according to which incomplete adenosine withdrawal in the brain upon awakening may be the cause of sleep inertia (Trotti, 2017; Van Dongen et al., 2001).

We did find very little significant differences in the within and between network functional connectivity at pre-sleep compared to 25 min post-awakening. Together with the absence of differences in the spectral and behavioral measures, this illustrates that the effects of sleep inertia have progressively dissipated over time and supports the dominant hypothesis of a typical duration of sleep inertia comprised between 20 and 30 min.

Contrary to what was expected, we did not observe a global modification of the DMN connectivity at awakening when comparing conditions within subjects (Fig. 5 *Top*). However, we did identify a negative correlation between the DMN functional connectivity (before and just after the nap) and the total duration of N2/N3 sleep (Fig. 6). The total duration of N2/N3 sleep was negatively correlated with the DMN functional connectivity at pre-nap (when participants were tired), and at 5 min post-awakening (during the maximum of sleep inertia), but not at 25 min post-awakening, when sleep inertia had dissipated. These results suggest that weak DMN functional connectivity could be a marker of sleep pressure, fatigue, or even the ability to sleep. This is consistent with previous studies reporting that decreased DMN connectivity is associated with daytime sleepiness (Ward et al., 2013) and is consistently found after sleep deprivation (De Havas et al., 2012).

We have also found that the DMN connectivity was positively correlated with the percentage of mistakes (Fig. S6). This is consistent with the idea that high DMN activation is associated with increased attentional lapses (Weissman et al., 2006) and task-related errors (Eichele et al., 2008).

4.4. Impact of the sleep stage preceding awakening

We found that neither the EEG spectral power nor the behavioral performance were significantly different between the participants who were awakened in N2 sleep and those who were awakened in N3 sleep. Regarding performance, the delay between the awakening and the DST (approximately 10 min) have prevented us from comparing the 2 groups at the maximum of sleep inertia which may explain the lack of significant difference between the groups. However, we did observe several between-group differences in the brain networks functional connectivity, which was assessed right after awakening. Overall, the pattern of functional disruption that followed awakening from N3 sleep was more pronounced than after awakening from N2 sleep. For instance, the significant interaction between group and time revealed that the reduced anti-correlation between the DMN and task-positive networks (DAN, salience, SM) was mainly driven by the N3 group (Fig. 5). These findings suggest that only awakening from N3 sleep is associated with a robust disruption in the brain networks functional connectivity. This is well in line with previous behavioral research on sleep inertia showing that the sleep stage prior to awakening is *"one of the most critical factors"*, with abrupt awakening during N3 sleep producing the most severe sleep inertia (Tassi and Muzet, 2000).

It is also noteworthy that, for the N2 group, the most visible changes in ROI-to-ROI connectivity were observed when contrasting the 25 min and 5 min post-awakening scans, whereas, for the N3 group, it was when contrasting the pre-nap and 5 min post-awakening scans. This observation suggests that the time course of sleep inertia dissipation could be faster in the N2 group than in the N3 group.

To summarize, the finding that the disruption of between networks functional connectivity at awakening was increased when the previous sleep was deeper supports our second hypothesis.

4.5. Limitations and perspectives

Due to our choice to maximize sleep inertia at awakening in the scanner by partially depriving participants from sleep the night before the fMRI session, they were therefore tired during the pre-nap scan. It follows that it is likely that the brain measures and behavioral performances during this scan were altered as compared to rested wakefulness after a full night of sleep (De Havas et al., 2012; Kaufmann et al., 2016; Sämann et al., 2010; Tüshaus et al., 2017; Yeo et al., 2015). However, we believe that this choice does not jeopardize the value of the results for two reasons. First, the most probable effect of the partial sleep deprivation on the night before is to reduce the magnitude of the difference between the pre-sleep and the first post-sleep measurements. It follows that the effect sizes of the physiological and behavioral differences between the pre-sleep and the first post-sleep measurements are likely underestimated compared to if the participants had been fully rested during the pre-sleep measurements. Future studies are required to assess the amplitude of the possible underestimation of the behavioral and physiological correlates of sleep inertia in our study. Second, our protocol reproduces ecological situations, indeed most of the workers concerned by the necessity to act/decide urgently at awakening (e.g. physicians, nurses, military) are typically sleep deprived (Weinger and Ancoli-Israel, 2002) and often take naps during extensive duty shifts to limit sleep pressure.

It is also possible that the MRI scan noise may have had a positive alerting effect on vigilance and therefore reduced the severity of sleep inertia. Only one study has so far investigated the effect of a 75 dB pink noise on sleep inertia and the authors reported inconclusive results on whether noise improved or worsened sleep inertia effects after a nap (Tassi et al., 1992).

Finally, it should be noted that the EEG cap used in this study was primarily designed for EEG-fMRI sleep studies (i.e. to allow sleep scoring while remaining comfortable for the participants) and as such did only contain a limited number of channels. Future studies using high-density EEG or magnetoencephalography (MEG) might provide more precise insights on the relationship between EEG spectral power and brain networks functional connectivity.

Among the perspectives for future studies, it would be interesting to extend these data to N1 sleep and REM sleep, which are known to induce less sleep inertia than N2 or N3 sleep (Tassi and Muzet, 2000). More broadly, future studies should aim at better delineating the brain networks functional connectivity changes specific to each sleep stage. Methodologically speaking, a promising avenue of research would be to use the recently developed technique of dynamic functional connectivity (Hutchison et al., 2013) to obtain a less static measure of the dissipation of sleep inertia across the first minutes after awakening. For example, by scanning continuously over the first half hour after awakening, one could then use a sliding windows approach to evaluate the temporal variations in functional connectivity across the first minutes following awakening.

5. Conclusion

Using combined behavioral, EEG and fMRI measurements, we provided an extensive overview of the cognitive and brain change after awakening from N2 and N3 sleep. Within-subject comparisons supported our first hypothesis: the first minutes after awakening from sleep are marked by a decrease in cognitive performances and the intrusion of sleep-specific brain activity, reflected mainly by a higher spectral power of slow activity and a loss of the functional segregation of brain networks. Furthermore, between-subject comparisons showed that only awakening from N3 sleep induced a robust loss of functional segregation, therefore providing evidence for our second hypothesis: the severity of the behavioral and brain alterations at awakening are linked to the previous sleep duration and depth.

Acknowledgements

This research was partly performed within the framework of the LabEx Cortex (ANR-11-LABX-0042) of Université de Lyon, within the program "Investissements d'avenir" (ANR-11-IDEX-0007) operated by the French National Research Agency (ANR).

The authors would like to thank Basak Turker, Morgane Hamon,

Franck Lamberton and Danielle Ibarrola for substantial help in data collection and analysis, as well as Jamila Lagha for her help in administrative work and Quentin Barthelemy for his valuable comments on the EEG spectral analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroimage.2018.09.033.

Author contribution

R.V and P.R designed the study and acquired the data. R.V analyzed the data with the help of D. M for fMRI data. RV wrote the article and PR participated in the writing process by thoroughly reviewing the manuscript. A.N helped in study conception and provided access to his sleep unit to conduct the sleep deprivation.

Conflicts of interest

The authors declare no conflict of interest.

Code availability

Custom Python scripts used for spectral, functional connectivity and behavioral analysis are available upon reasonable request.

References

Allen, P.J., Josephs, O., Turner, R., 2000. A method for removing imaging artifact from continuous EEG recorded during functional MRI. Neuroimage 12, 230–239.
Allen, P.J., Polizzi, G., Krakow, K., Fish, D.R., Lemieux, L., 1998. Identification of EEG events in the MR scanner: the problem of pulse artifact and a method for its subtraction. Neuroimage 8, 229–239.
Anderson, J.S., Ferguson, M.A., Lopez-Larson, M., Yurgelun-Todd, D., 2011. Reproducibility of single-subject functional connectivity measurements. AJNR Am. J. Neuroradiol. 32, 548–555.
Balkin, T.J., Braun, A.R., Wesensten, N.J., Jeffries, K., Varga, M., Baldwin, P., Belenky, G., Herscovitch, P., 2002. The process of awakening: a PET study of regional brain activity patterns mediating the re-establishment of alertness and consciousness. Brain 125, 2308–2319.
Benca, R.M., Obermeyer, W.H., Larson, C.L., Yun, B., Dolski, I., Kleist, K.D., Weber, S.M., Davidson, R.J., 1999. EEG alpha power and alpha power asymmetry in sleep and wakefulness. Psychophysiology 36, 430–436.
Behzadi, Y., Restom, K., Liau, J., Liu, T.T., 2007. A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. Neuroimage 37, 90–101.
Bruck, D., Pisani, D.L., 1999. The effects of sleep inertia on decision-making performance. J. Sleep Res. 8, 95–103.
Cantero, J.L., Atienza, M., Salas, R.M., 2002. Human alpha oscillations in wakefulness, drowsiness period, and REM sleep: different electroencephalographic phenomena within the alpha band. Neurophysiol. Clin. 32, 54–71.
Cole, R.J., Kripke, D.F., Gruen, W., Mullaney, D.J., Gillin, J.C., 1992. Automatic sleep/wake identification from wrist activity. Sleep 15, 461–469.
Combrisson, E., Vallat, R., Eichenlaub, J.-B., O'Reilly, C., Lajnef, T., Guillot, A., Ruby, P.M., Jerbi, K., 2017. Sleep: an open-source python software for visualization, analysis and staging of sleep data. Front. Neuroinf. 11, 60.
Davidson, R.J., Jackson, D.C., Larson, C.L., 2000. Human electroencephalography. Handbook of psychophysiology 2, 27–52.
De Havas, J.A., Parimal, S., Soon, C.S., Chee, M.W.L., 2012. Sleep deprivation reduces default mode network connectivity and anti-correlation during rest and task performance. Neuroimage 59, 1745–1751.
Dinges, D.F., Orne, M.T., Orne, E.C., 1985. Assessing performance upon abrupt awakening from naps during quasi-continuous operations. Behav. Res. Methods Instrum. Comput. 17, 37–45.
Drapeau, C., Carrier, J., 2004. Fluctuation of waking electroencephalogram and subjective alertness during a 25-hour sleep-deprivation episode in young and middle-aged subjects. Sleep 27, 55–60.
Duyn, J.H., 2012. EEG-fMRI methods for the study of brain networks during sleep. Front. Neurol. 3. https://doi.org/10.3389/fneur.2012.00100.
Eichele, T., Debener, S., Calhoun, V.D., Specht, K., Engel, A.K., Hugdahl, K., von Cramon, D.Y., Ullsperger, M., 2008. Prediction of human errors by maladaptive changes in event-related brain networks. Proc. Natl. Acad. Sci. U.S.A. 105, 6173–6178.
Evans, F.J., Orne, M.T., 1975. Recovery from Fatigue. DTIC Document.
Faraut, B., Andrillon, T., Vecchierini, M.-F., Leger, D., 2017. Napping: a public health issue. From epidemiological to laboratory studies. Sleep Med. Rev. 35, 85–100. https://doi.org/10.1016/j.smrv.2016.09.002.
Ferrara, M., Curcio, G., Fratello, F., Moroni, F., Marzano, C., Pellicciari, M., Gennaro, L., 2006. The electroencephalographic substratum of the awakening. Behav. Brain Res. 167, 237–244.
Finn, E.S., Shen, X., Scheinost, D., Rosenberg, M.D., Huang, J., Chun, M.M., Papademetris, X., Constable, R.T., 2015. Functional connectome fingerprinting: identifying individuals using patterns of brain connectivity. Nat. Neurosci. 18, 1664–1671. https://doi.org/10.1038/nn.4135.
Gorgoni, M., Ferrara, M., D'Atri, A., Lauri, G., Scarpelli, S., Truglia, I., De Gennaro, L., 2015. EEG topography during sleep inertia upon awakening after a period of increased homeostatic sleep pressure. Sleep Med. 16, 883–890.
Gramfort, A., Luessi, M., Larson, E., Engemann, D.A., Strohmeier, D., Brodbeck, C., Parkkonen, L., Hämäläinen, M.S., 2014. MNE software for processing MEG and EEG data. Neuroimage 86, 446–460. https://doi.org/10.1016/j.neuroimage.2013.10.027.
Horne, J., Moseley, R., 2011. Sudden early-morning awakening impairs immediate tactical planning in a changing "emergency" scenario. J. Sleep Res. 20, 275–278.
Horovitz, S.G., Braun, A.R., Carr, W.S., Picchioni, D., Balkin, T.J., Fukunaga, M., Duyn, J.H., 2009. Decoupling of the brain's default mode network during deep sleep. Proc. Natl. Acad. Sci. Unit. States Am. 106, 11376–11381.
Hutchison, R.M., Womelsdorf, T., Allen, E.A., Bandettini, P.A., Calhoun, V.D., Corbetta, M., Della Penna, S., Duyn, J.H., Glover, G.H., Gonzalez-Castillo, J., Handwerker, D.A., Keilholz, S., Kiviniemi, V., Leopold, D.A., de Pasquale, F., Sporns, O., Walter, M., Chang, C., 2013. Dynamic functional connectivity: promise, issues, and interpretations. Neuroimage 80, 360–378.
Iber, C., 2007. The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications. American Academy of Sleep Medicine.
Kaufmann, T., Elvsåshagen, T., Alnæs, D., Zak, N., Pedersen, P.Ø., Norbom, L.B., Quraishi, S.H., Tagliazucchi, E., Laufs, H., Bjørnerud, A., Malt, U.F., Andreassen, O.A., Roussos, E., Duff, E.P., Smith, S.M., Groote, I.R., Westlye, L.T., 2016. The brain functional connectome is robustly altered by lack of sleep. Neuroimage 127, 324–332. https://doi.org/10.1016/j.neuroimage.2015.12.028.
Krause, A.J., Simon, E.B., Mander, B.A., Greer, S.M., Saletin, J.M., Goldstein-Piekarski, A.N., Walker, M.P., 2017. The sleep-deprived human brain. Nat. Rev. Neurosci. 18, 404–418.
Kirkwood, Thomas BL., 1979. Geometric Means and Measures of Dispersion, pp. 908–909.
Larson-Prior, L.J., Power, J.D., Vincent, J.L., Nolan, T.S., Coalson, R.S., Zempel, J., Snyder, A.Z., Schlaggar, B.L., Raichle, M.E., Petersen, S.E., 2011. Modulation of the brain's functional network architecture in the transition from wake to sleep. Prog. Brain Res. 193, 277–294. https://doi.org/10.1016/B978-0-444-53839-0.00018-1.
Marzano, C., Ferrara, M., Moroni, F., De Gennaro, L., 2011. Electroencephalographic sleep inertia of the awakening brain. Neuroscience 176, 308–317. https://doi.org/10.1016/j.neuroscience.2010.12.014.
McKinney, S.M., Dang-Vu, T.T., Buxton, O.M., Solet, J.M., Ellenbogen, J.M., 2011. Covert waking brain activity reveals instantaneous sleep depth. PLoS One 6 e17351. https://doi.org/10.1371/journal.pone.0017351.
Mueller, S., Wang, D., Fox, M.D., Yeo, B.T.T., Sepulcre, J., Sabuncu, M.R., Shafee, R., Lu, J., Liu, H., 2013. Individual variability in functional connectivity architecture of the human brain. Neuron 77, 586–595. https://doi.org/10.1016/j.neuron.2012.12.028.
Ogilvie, R.D., Simons, I., 1992. Falling asleep and waking up: a comparison of EEG spectra. Sleep, arousal and performance 73–87.
Pfurtscheller, G., Stancak Jr., A., Neuper, C., 1996. Event-related synchronization (ERS) in the alpha band—an electrophysiological correlate of cortical idling: a review. Int. J. Psychophysiol. 24, 39–46.
Picchioni, D., Duyn, J.H., Horovitz, S.G., 2013. Sleep and the functional connectome. Neuroimage 80, 387–396.
Sämann, P.G., Tully, C., Spoormaker, V.I., Wetter, T.C., Holsboer, F., Wehrle, R., Czisch, M., 2010. Increased sleep pressure reduces resting state functional connectivity. Magn. Reson. Mater. Phys. Biol. Med. 23, 375–389.
Sämann, P.G., Wehrle, R., Hoehn, D., Spoormaker, V.I., Peters, H., Tully, C., Holsboer, F., Czisch, M., 2011. Development of the brain's default mode network from wakefulness to slow wave sleep. Cerebr. Cortex 21, 2082–2093. https://doi.org/10.1093/cercor/bhq295.
Silber, M.H., Ancoli-Israel, S., Bonnet, M.H., Chokroverty, S., Grigg-Damberger, M.M., Hirshkowitz, M., Kapen, S., Keenan, S.A., Kryger, M.H., Penzel, T., Pressman, M.R., Iber, C., 2007. The visual scoring of sleep in adults. J. Clin. Sleep Med. 3, 121–131.
Slater, J.A., Botsis, T., Walsh, J., King, S., Straker, L.M., Eastwood, P.R., 2015. Assessing sleep using hip and wrist actigraphy: hip and wrist actigraphy. Sleep Biol. Rhythm 13, 172–180.
Smith, S.M., Beckmann, C.F., Andersson, J., Auerbach, E.J., Bijsterbosch, J., Douaud, G., Duff, E., Feinberg, D.A., Griffanti, L., Harms, M.P., Kelly, M., Laumann, T., Miller, K.L., Moeller, S., Petersen, S., Power, J., Salimi-Khorshidi, G., Snyder, A.Z., Vu, A.T., Woolrich, M.W., Xu, J., Yacoub, E., Uğurbil, K., Van Essen, D.C., Glasser, M.F., Minn HCP Consortium, W.U., 2013. Resting-state fMRI in the human connectome project. Neuroimage 80, 144–168.
Soares, J.M., Magalhães, R., Moreira, P.S., Sousa, A., Ganz, E., Sampaio, A., Alves, V., Marques, P., Sousa, N., 2016. A hitchhiker's guide to functional magnetic resonance imaging. Front. Neurosci. 10, 515.
Spoormaker, V.I., Gleiser, P.M., Czisch, M., 2012. Frontoparietal connectivity and hierarchical structure of the brain's functional network during sleep. Front. Neurol. 3. https://doi.org/10.3389/fneur.2012.00080.
Stampi, C., Mullington, J., Rivers, M., Campos, J.P., Broughton, R., 1990. Ultrashort sleep schedules: sleep architecture and the recuperative value of multiple 80- 50- and 20-min naps, in: sleep 90. J. Horne, Bochum 71–74.
Tassi, P., Muzet, A., 2000. Sleep inertia. Sleep Med. Rev. 4, 341–353.

Tassi, P., Nicolas, A., Dewasmes, G., Eschenlauer, R., Ehrhart, J., Salame, P., Muzet, A., Libert, J.P., 1992. Effects of noise on sleep inertia as a function of circadian placement of a one-hour nap. Percept. Mot. Skills 75, 291–302.

Trotti, L.M., 2017. Waking up is the hardest thing I do all day: sleep inertia and sleep drunkenness. Sleep Med. Rev. 35, 76–84. https://doi.org/10.1016/j.smrv.2016.08.005.

Tsai, P.-J., Chen, S.C.-J., Hsu, C.-Y., Wu, C.W., Wu, Y.-C., Hung, C.-S., Yang, A.C., Liu, P.-Y., Biswal, B., Lin, C.-P., 2014. Local awakening: regional reorganizations of brain oscillations after sleep. Neuroimage 102, 894–903.

Tudor-Locke, C., Barreira, T.V., Schuna Jr., J.M., Mire, E.F., Katzmarzyk, P.T., 2014. Fully automated waist-worn accelerometer algorithm for detecting children's sleep-period time separate from 24-h physical activity or sedentary behaviors. Appl. Physiol. Nutr. Metabol. 39, 53–57.

Tüshaus, L., Balsters, J.H., Schläpfer, A., Brandeis, D., O'Gorman Tuura, R., Achermann, P., 2017. Resisting sleep pressure: impact on resting state functional network connectivity. Brain Topogr. 30, 757–773. https://doi.org/10.1007/s10548-017-0575-x.

Vallat, R., Eskinazi, M., Nicolas, A., Ruby, P., 2018. Sleep and dream habits in a sample of French college students who report no sleep disorders. J. Sleep Res. 27, e12659. https://doi.org/10.1111/jsr.12659.

Vallat, R., Lajnef, T., Eichenlaub, J.-B., Berthomier, C., Jerbi, K., Morlet, D., Ruby, P.M., 2017. Increased evoked potentials to arousing auditory stimuli during sleep: implication for the understanding of dream recall. Front. Hum. Neurosci. 11. https://doi.org/10.3389/fnhum.2017.00132.

Van Dijk, K.R.A., Hedden, T., Venkataraman, A., Evans, K.C., Lazar, S.W., Buckner, R.L., 2010. Intrinsic functional connectivity as a tool for human connectomics: theory, properties, and optimization. J. Neurophysiol. 103, 297–321.

Van Dongen, H.P., Price, N.J., Mullington, J.M., Szuba, M.P., Kapoor, S.C., Dinges, D.F., 2001. Caffeine eliminates psychomotor vigilance deficits from sleep inertia. Sleep 24, 813–819.

Ward, A.M., McLaren, D.G., Schultz, A.P., Chhatwal, J., Boot, B.P., Hedden, T., Sperling, R.A., 2013. Daytime sleepiness is associated with decreased default mode network connectivity in both young and cognitively intact elderly subjects. Sleep 36, 1609–1615.

Weinger, M.B., Ancoli-Israel, S., 2002. Sleep deprivation and clinical performance. J. Am. Med. Assoc. 287, 955–957.

Weissenbacher, A., Kasess, C., Gerstl, F., Lanzenberger, R., Moser, E., Windischberger, C., 2009. Correlations and anticorrelations in resting-state functional connectivity MRI: a quantitative comparison of preprocessing strategies. Neuroimage 47, 1408–1416.

Weissman, D.H., Roberts, K.C., Visscher, K.M., Woldorff, M.G., 2006. The neural bases of momentary lapses in attention. Nat. Neurosci. 9, 971–978.

Whitfield-Gabrieli, S., Nieto-Castanon, A., 2012. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. Brain Connect. 2, 125–141.

Whitlow, C.T., Casanova, R., Maldjian, J.A., 2011. Effect of resting-state functional MR imaging duration on stability of graph theory metrics of brain network connectivity. Radiology 259, 516–524.

Wong, C.W., Olafsson, V., Tal, O., Liu, T.T., 2013. The amplitude of the resting-state fMRI global signal is related to EEG vigilance measures. Neuroimage 83, 983–990.

Wu, C.W., Liu, P.-Y., Tsai, P.-J., Wu, Y.-C., Hung, C.-S., Tsai, Y.-C., Cho, K.-H., Biswal, B.B., Chen, C.-J., Lin, C.-P., 2012. Variations in connectivity in the sensorimotor and default-mode networks during the first nocturnal sleep cycle. Brain Connect. 2, 177–190.

Yeo, B.T.T., Tandi, J., Chee, M.W.L., 2015. Functional connectivity during rested wakefulness predicts vulnerability to sleep deprivation. Neuroimage 111, 147–158.