Research Articles: Systems/Circuits

Human NREM Sleep Promotes Brain-Wide Vasomotor and Respiratory Pulsations

<https://doi.org/10.1523/JNEUROSCI.0934-21.2022>

Cite as: J. Neurosci 2022; 10.1523/JNEUROSCI.0934-21.2022

Received: 1 May 2021

Revised: 10 January 2022

Accepted: 11 January 2022

This Early Release article has been peer-reviewed and accepted, but has not been through the composition and copyediting processes. The final version may differ slightly in style or formatting and will contain links to any extended data.

Alerts: Sign up at www.jneurosci.org/alerts to receive customized email alerts when the fully formatted version of this article is published.

Copyright © 2022 Helakari et al.

This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license, which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.

1 **Human NREM sleep promotes brain-wide vasomotor and respiratory pulsations**

2 Abbreviated title: NREM sleep promotes physiological brain pulsations

3 **Helakari H^{1,2,3}, Korhonen V^{1,2,3}, Holst SC⁴, Piispala J^{2,3,5}, Kallio M^{2,3,5}, Väyrynen T^{1,2,3}, Huotari N^{1,2,3},**
 4 **Raitamaa L^{1,2,3}, Tuunanen J^{1,2,3}, Kananen J^{1,2,3}, Järvelä M^{1,2,3}, Tuovinen T^{1,2,3}, Raatikainen V^{1,2,3}, Borchardt**
 5 **V^{1,2,3}, Kinnunen H⁶, Nedergaard M^{7,8} and Kiviniemi V^{1,2,3}**

6 **Affiliations**

7 ^{1*} Oulu Functional Neuroimaging (OFNI), Department of Diagnostic Radiology, Oulu University Hospital, 90220
 8 Oulu, Finland

9 ² Medical Imaging, Physics and Technology (MIPT), Faculty of Medicine, University of Oulu, 90220 Oulu,
 10 Finland

11 ³ Medical Research Center (MRC), 90220 Oulu, Finland

12 ⁴ Neurobiology Research Unit, University of Copenhagen, 2200 Copenhagen, Denmark

13 ⁵ Clinical Neurophysiology, Oulu University Hospital, 90220 Oulu, Finland

14 ⁶ Oura Health Ltd.'s, 90590 Oulu, Finland

15 ⁷ Center of Translational Neuromedicine, University of Copenhagen, 2200 Copenhagen, Denmark

16 ⁸ Center of Translational Neuromedicine, University of Rochester, 14642 Rochester NY, US

17 ^{*} The work was performed in Oulu University Hospital

18 **Corresponding Author**

19 Vesa Kiviniemi, Prof, MD

20 vesa.kiviniemi@oulu.fi

21 Department of Diagnostic Radiology, Medical Research Center (MRC), Oulu University Hospital, Kajaanintie 50,
 22 90220, Oulu

23 Heta Helakari, MSc

24 heta.helakari@oulu.fi

25 Department of Diagnostic Radiology, Medical Research Center (MRC), Oulu University Hospital, Kajaanintie 50,
26 90220, Oulu

27 Number of figures: 6

28 Number of tables: 2

29 Number of words for abstract: 264

30 Number of words for introduction: 752

31 Number of words for discussion: 1594

32 **Limitations and Conclusions requested by both reviewers:**

33 Discussion (if limitations included): 1837

34 Number of words for Limitations: 242

35 Number of words for Conclusions: 109

36 **Conflict of interest**

37 Financial Disclosure: none

38 Non-financial Disclosure: none

39 Oura Health Ltd.'s provided the surveillance smart rings for the study, but did not participate in the analysis. The
40 manuscript in question is published as a preprint in bioRxiv, but we guarantee that it does not infringe any
41 subsequent copyright or license agreement.

42 **Acknowledgements**

43 This work was supported by Uniogs/MRC Oulu DP-grant (HH, JK), Pohjois-Suomen Terveysthuollon tukisäätiö
44 (HH, VKo), JAES-Foundation (VKi), Academy of Finland and Aivosäätiö TERVA grant 314497 (VKi), Academy
45 of Finland Grant 275342 (VKi), The SalWe Research Program for Mind and Body (Tekes—the Finnish Funding
46 Agency for Technology and Innovation, Grant No. 1104/10) (VKi), The Finnish Medical Foundation (VKi, JK,
47 TT), Finnish Neurological Foundation (VKi), KEVO grants from Oulu University hospital (VKi), Orion Research
48 Foundation sr (JK, TT), Medical Research Center (MRC) –Oulu (JK, VR), Maire Taponen Foundation sr (JK),
49 Finnish Brain Foundation sr (JK, VKi), Instrumentarium Science Foundation sr (JK), The University of Oulu
50 Scholarship Foundation (JK, VR).

51 We would like to thank all study subjects for their participation in the study. We also thank Tuomas Konttajärvi for
52 assistance in measurements and preprocessing of EEG data, Jani Häkli, Annastiina Kivipää, Tarja Holtinkoski,
53 Aleksi Rasila, Taneli Hautaniemi, Miia Lampinen and others who assisted in measurements or otherwise
54 contributed. We are grateful for devices and data provided by Oura. We wish to acknowledge Jussi Kantola for data
55 management and reconstruction of MREG data, the CSC – IT Center for Science Ltd., Finland for generous
56 computational resources, Prof. Paul Cumming of Bern University Hospital for comments on the manuscript, and
57 Dan Xue for assistance with the figures.

58

59

60

61 **Abstract**

62 The physiological underpinnings of the necessity of sleep remains uncertain. Recent evidence suggests that sleep
63 increases the convection of cerebrospinal fluid (CSF) and promotes the export of interstitial solutes, thus providing
64 a framework to explain why all vertebrate species require sleep. Cardiovascular, respiratory, and vasomotor brain
65 pulsations have each been shown to drive CSF flow along perivascular spaces, yet it is unknown how such
66 pulsations may change during sleep in humans. To investigate these pulsation phenomena in relation to sleep, we
67 simultaneously recorded fast fMRI, magnetic resonance encephalography (MREG) and electroencephalography
68 (EEG) signals in a group of healthy volunteers. We quantified sleep-related changes in the signal frequency
69 distributions by spectral entropy analysis and calculated the strength of the physiological (vasomotor, respiratory,
70 and cardiac) brain pulsations by power sum analysis in 15 subjects (aged 26.5 ± 4.2 years, 6 females). Finally, we
71 identified spatial similarities between EEG slow oscillation (0.2-2 Hz) power and MREG pulsations. Compared to
72 wakefulness, non-rapid eye movement (NREM) sleep was characterized by reduced spectral entropy and increased
73 brain pulsation intensity. These effects were most pronounced in posterior brain areas for very low frequency (≤ 0.1
74 Hz) vasomotor pulsations but were also evident brain-wide for respiratory pulsations, and to a lesser extent for
75 cardiac brain pulsations. There was increased EEG slow oscillation power in brain regions spatially overlapping
76 with those showing sleep-related MREG pulsation changes. We suggest that reduced spectral entropy and enhanced
77 pulsation intensity are characteristic of NREM sleep; together with our findings of increased power of slow
78 oscillation, present results support the proposition that sleep promotes fluid transport in human brain.

79 **Statement of Significance**

80 We report that the spectral power of physiological brain pulsation mechanisms driven by vasomotor, respiration
81 and cardiac rhythms in human brain increase during sleep, extending previous observations of their association
82 with glymphatic brain clearance during sleep in rodents. The magnitudes of increased pulsations follow the rank
83 order vasomotor > respiratory > cardiac pulsations, with correspondingly declining spatial extents. Spectral
84 entropy, previously known as vigilance and as an anesthesia metric, decreased during NREM sleep compared with
85 the awake state in very low and respiratory frequencies, indicating reduced signal complexity. An EEG slow
86 oscillation power increase occurring in the early sleep phase (NREM 1-2) spatially overlapped with pulsation
87 changes, indicating reciprocal mechanisms between those measures.

88 Introduction

89 A notable feature of the mammalian brain is that it consists of almost 80% water (Tait et al. 2008), with a near
90 absence of connective tissue. Also, the brain floats in cerebrospinal fluid (CSF) within the closed cavity of the
91 skull. These physical characteristics enable brain-wide pulsations that drive fast fluid movement within the
92 neuropil. In the early 20th century Hans Berger described three forms of brain pressure pulsations: “eine
93 pulsatorische, eine respiratorische und vasomotorische Bewegung” (Berger 1901); his studies of pulsatile brain
94 activity lead the invention of electroencephalography (EEG).

95 Nearly a century later, the same triad of cardiovascular, respiratory and slow, spontaneous vasomotor waves driven
96 by arteries can be non-invasively imaged throughout the brain by magnetic resonance encephalography (MREG),
97 which is a fast variant of functional magnetic resonance imaging (fMRI) (Kiviniemi et al. 2016) designed to
98 maximize temporal accuracy while maintaining adequate image quality. Besides MREG, several fMRI imaging
99 techniques have shown distinct cardiac, respiratory (Dreha-Kulaczewski et al. 2015; Klose et al. 2000; Matsumae et
100 al. 2019; Posse et al. 2013; Sloots et al. 2020; Yamada et al. 2013) and very low frequency (<0.1 Hz) (Fultz et al.
101 2019) pulsations in the living human brain. The pulsations must bear some relation with the normal resting heart
102 rate, which ranges between 1-1.67 Hz in humans (Mason et al. 2007) and respiratory rate, which is within or close
103 to range 0.16–0.33 Hz (Russo et al. 2017); both of these rates decrease during non-rapid eye movement (NREM
104 sleep) (Penzel et al. 2003). Compared to other techniques, MREG enables robust detection and separation of
105 physiological brain pulsations in the whole brain due to the greater spectral resolution in the range of 0-5 Hz, which
106 is obtained without signal aliasing (Huotari et al. 2019; Raitamaa et al. 2018; Tuovinen et al. 2020).

107 Sleep is known to elicit electrohydrodynamic changes in brain tissue (Ding et al. 2016) that are driven by the
108 physiological pulsations (Mestre et al. 2018). Also, prior reports in rodents showed that natural sleep and certain
109 anesthetic regimens can induce a sharp increase in the influx of CSF to brain tissue, which further announce an
110 increase in slow wave electrophysiological activity (Hablitz et al. 2019; Xie et al. 2013). A recent study in mice
111 associated 0.1 Hz vasomotor wave activity with increased paravascular solute transport (van Veluw et al. 2020),
112 thus serving as an additional driver of fluid convection supplementing the previously presented cardiovascular
113 brain pulsatility (Iliff et al. 2013; Mestre et al. 2018). In humans, NREM sleep and drowsiness are associated with
114 high amplitude of very low frequency fluctuations of blood-oxygen-level-dependent (BOLD) signal (Chang et al.

2016; Fukunaga et al. 2006; Horovitz et al. 2008; Wong et al. 2016), which are suggested to arise from vasomotion (Biswal et al. 1995; Kiviniemi et al. 2000; Rayshubskiy et al. 2014; Silvani et al. 2004; Wang et al. 2008). Furthermore, simultaneous EEG-fMRI study in humans linked increased delta wave amplitude during sleep with decreased cerebral blood volume followed by increased flow of CSF into the fourth ventricle (Fultz et al. 2019). Also, the previous human neuroimaging literature suggests that respiratory pulsation is the main pulsatory driver of CSF flow and intracerebral venous blood flow (Dreha-Kulaczewski et al. 2015; Matsumae et al. 2019; Vinje et al. 2019). Based on these various findings, we concluded that brain pulsations may affect the influx of CSF in a manner related to the state of consciousness and in association with slow wave electrophysiological activity.

Based on this scenario, we used whole brain MREG with synchronous EEG sleep monitoring during wakefulness and sleep to test the hypotheses that MREG pulsations change during sleep, and that the pulsations occur in association with EEG slow oscillations. By inducing a T2* MRI signal, MREG allows us to study brain fluid dynamics originating not only from blood oxygenation (i.e. susceptibility related BOLD effect), but also the dynamics arising from the CSF space and interstitial fluid changes. The short repetition time (TR 100 ms) and the used read pulse flip angle (FA 5°), both increase inflow effects and sensitize detection of the steady state precession alterations induced in the brain MRI data by relatively sudden cardiorespiratory impulses. We studied spectral entropy and the power sum of the MREG signal to assess sleep-related pulsation changes and demonstrated their relation to EEG slow oscillations (0.02-2 Hz) with spatial co-localization. Overall, we found significant changes in vasomotor, respiratory, and cardiac pulsations during NREM sleep compared to awake, mostly in brain areas overlapping with those showing EEG slow oscillation changes.

Materials and Methods

Subjects

Twenty-five subjects (aged 28.0 ± 5.9 years, 11 females) participated in the study, which was approved by the Regional Ethics Committee of the Northern Ostrobothnia Hospital District. Written informed consent was obtained from all participants, according to requirements of the Declaration of Helsinki. Subjects were recruited from among university students by advertisement. Inclusion criteria for the study were sent via e-mail to all subjects, who were then interviewed to screen out cases meeting exclusion criteria. All subjects were healthy as assessed by the

141 interview and met the following inclusion criteria: no continuous medication, no neurological or cardio-respiratory
142 diseases, non-smokers and no pregnancy. We did not undertake screening for sleep apnea or other sleep disorders.
143 The subjects were instructed to not consume caffeine four hours preceding the Awake scan session and eight hours
144 preceding the Sleep scan session. Subjects were further instructed to abstain from alcohol during the night of sleep
145 deprivation. Sample sizes of 15 (aged 26.5 ± 4.2 years, 6 females) and 12 (26.2 ± 4.3 , 5 females) were used for the
146 main analysis (Figure 1).

147 **Insert Figure 1 about here.**

148 **Data collection**

149 Subjects were scanned after a normal night's sleep (7.8 ± 1.3 hours sleep the previous night) in the afternoon
150 starting at 4-6 PM (and three days later after a monitored interval of wakefulness lasting 24 ± 1.3 hours) in the early
151 morning starting at 6-8 AM (Figure 2a). We had sleep deprived the subjects aiming to maximize the likelihood of
152 the subjects entering a deeper and more consolidated sleep state during the next day's MR imaging session
153 (Horovitz et al. 2009; Kaufmann et al. 2006). At home, the sleep deprivation was verified by a smart ring (see
154 details below). Subjects were instructed to wear the ring for at least 24 h preceding the Awake and Sleep scan
155 sessions. The smart ring records upper limb motion (3-D accelerometer, 50 Hz), photoplethysmogram (250 Hz) and
156 skin temperature (1/min). Validation studies for polysomnography showed 96% sensitivity for detecting sleep, 48%
157 specificity for detecting awake state, and medical-grade actigraphy ($r=0.86$; correlation in total sleep time), thus
158 confirming that the ring signals adequately separate awake from sleep states (de Zambotti et al. 2017; Mehrabadi et
159 al. 2020). Smart ring data were available for 14 of 15 scanning subjects.

160 All subjects were scanned in Oulu (Finland) using a Siemens MAGNETOM Skyra 3T (Siemens Healthineers AG,
161 Erlangen, Germany) scanner with a 32-channel head coil. The subjects were scanned with the MREG fast fMRI
162 sequence, in synchrony with a previously described multimodal scanning setup (Korhonen et al. 2014). MREG is a
163 single-shot sequence undersampling k-space with an in/out stack-of-spiral trajectories in three dimensions
164 (Assländer et al. 2013; Lee et al. 2013; Zahneisen et al. 2012). The following parameters were used for MREG:
165 repetition time (TR = 100 ms), echo time (TE = 36 ms), and flip angle (FA = 5°), field of view (FOV = 192 mm)
166 and 3 mm cubic voxels. Parameters for three-dimensional structural T1 MPRAGE were TR = 1900 ms, TE = 2.49
167 ms, FA = 9° , FOV = 240 mm, and slice thickness 0.9 mm. MREG data were reconstructed using L2-Tikhonov

168 regularization with lambda 0.1, where the latter regularization parameter was determined by the L-curve method
 169 with a MATLAB recon-tool provided by the sequence developers (Hugger et al. 2011).

170 Ear plugs were used to reduce perception of scanner noise, while head cushions restricted movement. During the
 171 Awake scan session, two MREG scans with simultaneous EEG and cardiorespiratory signals were recorded: I) ten-
 172 minute resting state, Eyes open, Awake scan (A_{1-2}) fixating on a cross on the screen, II) five-minute resting state,
 173 Eyes closed (EC) (Figure 2a) with ambient light. Three days later during the Sleep scan session, one or two MREG
 174 sequences were recorded: I) the first ten-minute Sleep scan (S_{1-2}), II) the second ten-minute Sleep scan (S_{3-4}),
 175 without ambient light during which the subjects were allowed to fall asleep *ad libitum*. Subjects were advised to
 176 contact the staff if they were not feeling somnolent after the first scan, which resulted in termination of the session
 177 for two of 15 subjects. An anatomical MR scan was performed at the end of both sessions.

178 When the subject was asleep when the scanning ended, lights were turned on and the subject was slowly woken up
 179 while remaining in the scanner. Due to individual differences in awake vigilance and ability to sleep in the MRI
 180 scanner, the ten-minute scans were divided into five-minute segments (A_1 , A_2 , S_1 , S_2 , S_3 and S_4) to separate the
 181 awake and sleep states more effectively (Figure 2a). We used five-minute segments because the accuracy of
 182 spectral analysis of very low frequency pulsations increases as a function of the data recording length. For further
 183 analysis, we selected the segment (from S_1 - S_4) with the highest sleep content, such that the Sleep data used for
 184 comparisons contained $91 \pm 11\%$ N1 or N2 sleep (A_1 versus S_n).

185 EEG (0.01 Hz high-pass filtering) was recorded using the Electrical Geodesics (EGI, Magstim Company Ltd,
 186 Whitland, UK) MR-compatible GES 400 system, with a 256-channel high density net. Electrode impedances were
 187 $<50 \text{ k}\Omega$ and the sampling rate 1 kHz (data from six subjects was sampled with 250 Hz by mistake, from which two
 188 (one A_{1-2} and one EC) EEG data sets were not usable for sleep scoring). Prior to scanning, signal quality was tested
 189 outside the scanner room by recording 30 second epochs of EEG with eyes open and eyes closed and by visually
 190 checking all the channels. Respiratory belt and fingertip peripheral SpO_2 and anesthesia monitor data (ECG,
 191 fingertip peripheral SpO_2 and end-tidal carbon dioxide (EtCO_2), Datex-Ohmeda S/5 Collect software) were
 192 measured in synchrony with the EEG, as described previously (Korhonen et al. 2014).

193 Prior to the imaging, the subjects performed the reaction time and paired-associate learning tests from CANTAB,
 194 Cambridge Neuropsychological Test Automated Battery, Cambridge, UK (CANTAB® 2019) on a tablet computer.
 195 The CANTAB test is a widely accepted standard for measuring cognitive state and vigilance level.

196 **Preprocessing and analysis of MREG data**

197 After reconstruction, MREG data were preprocessed and analyzed using FSL (5.09 BET software (Jenkinson et al.
 198 2012; Smith 2002)), AFNI (Analysis of Functional NeuroImages, v2 (Cox 1996)) and MATLAB (R2019). The
 199 brain was extracted from structural 3D MPRAGE volumes using neck clean-up and bias field correction options
 200 (Smith 2002). The functional data preprocessing was performed using the FSL pipeline. Data were high-pass
 201 filtered with a cut-off frequency of 0.008 Hz, and framewise head motion was then performed with FSL 5.08
 202 MCFLIRT software (Jenkinson et al. 2002). MCFLIRT relative mean displacement values (mm) of scan segments
 203 (A_1 versus EC, S_1 , S_2 , S_3 and S_4), Awake versus Sleep analysis (A_1 versus S_n) did not differ significantly (paired t-
 204 test, $p_{all} > 0.11$). Spatial smoothing was performed with *fslmaths* using a 5 mm FWHM Gaussian kernel. The
 205 highest spikes in the MREG data time series were removed using the *3dDespike* function in AFNI. Data were then
 206 registered into the Montreal Neurological Institute (MNI152) space at 3-mm resolution for comparable analysis
 207 between subjects. One hundred time points (10 seconds each) were excluded from the beginning of both five and
 208 ten-minute data segments so as to reach steady state signal saturation. Accordingly, from the ten-minute data, the
 209 final 100 time points were extracted to get a time series of the same length. Finally, we generated data sets for 2861
 210 time points (A_1 - S_4), which corresponds to approximately five minutes.

211 **Preprocessing and analysis of EEG data**

212 We employed standard EEG clean-up strategies and EEG sleep state scoring for datasets of the 15 subjects in
 213 whom we had simultaneously measured EEG and MREG. Due to instances of noisy EEG signals that were
 214 unsuitable for sleep scoring in three subjects, the final sample size in most of the MREG analysis was 12 (paired t-
 215 test, A_1 versus S_n). In EEG slow oscillation analysis, sample size was ten versus 12, because of 2/12 had no usable
 216 awake EEG. Data of all 15 subjects were used for calculating the accuracy of spectral entropy sleep detection (see
 217 next section).

218 EEG recordings were preprocessed using the Brain Vision Analyzer (Version 2.1; Brain Products) after file format
 219 conversion via BESA Research (Version 7.0). Gradient artifacts due to static and dynamic magnetic fields present

220 during the MRI data acquisition and ballistocardiographic (BCG) artifacts were corrected using the average artifact
 221 subtraction method (Allen et al. 1998; Allen et al. 2000). The absence of gradient of BCG artifacts was verified by
 222 visual inspection. After preprocessing, EEG data were visualized according to the 10-20 system instructions for
 223 sleep state scoring. Two experienced specialists in clinical neurophysiology, who were trained in EEG sleep
 224 scoring performed the scoring in 30 second epochs according to AASM guidelines for clinical sleep studies
 225 (American Academy of Sleep Medicine, 2017), and the final decisions on sleep states were obtained by consensus.
 226 EEG epochs were scored as awake, N1 (light sleep), N2 (intermediate sleep with sleep spindles and/or K-
 227 complexes), N3 (slow wave sleep) or REM (sleep with rapid eye movements).

228

229 For slow oscillation power spectral analysis, we removed linear trends to exclude stable conductivity
 230 changes at the electrode-skin interface, which could otherwise have affected the performance of various processing
 231 steps. We used fast independent component analysis (FastICA) in combination with principal component analysis
 232 (PCA) dimension reduction (150 components) to transform the datasets into independent components, where
 233 artificial ICs were identified and removed with the help of EEGLAB SASICA-tool after visual confirmation. To
 234 furthermore minimize non-linearities and enhance the performance of ICA, we used a spike detection algorithm to
 235 remove the most prominent artifactual signal excursions. To keep the recordings intact, we interpolated the trends
 236 for the gaps and used data from intact signal epochs to fill the gap in the manner similar
 237 to inpainting. Bad channels removed prior to ICA were afterwards reintroduced using spherical interpolation.
 238 Recordings were referenced to linked mastoid electrodes, which were located near to other electrodes but record
 239 less brain activity. Next, we generated five-minute segments (A_1 - S_4) corresponding with the analyzed MREG
 240 data. Power estimates were then calculated using short-time Fourier transform combined with a Hamming
 241 window of 30 seconds length and 50% overlap. We calculated relative power to attenuate the subject specific
 242 variability $RP_{sw} = P_{sw}/P_{tot}$. The time average was used to gain one RP estimate for each subject. As previous studies
 243 have linked increased CSF flow to slow wave changes (Fultz et al. 2019), we chose to study the slow wave activity
 244 (SWA, 0.5-4 Hz) and slow oscillations (0.2-2 Hz). Our direct current EEG allowed us to evaluate frequencies < 0.5
 245 Hz. To follow the previous literature, we set 0.2 Hz as the lower limit for slow oscillations, as these are known to
 246 increase in amplitude during N2 sleep (Brancaccio et al. 2020). The upper limit was chosen to meet
 247 the standard requirement in scoring of slow wave sleep.

248 **MREG signal spectral entropy analysis**

249 Spectral entropy from EEG and EMG is known to measure depth of anesthesia (Viertiö-Oja et al. 2004) and sleep
 250 (Mahon et al. 2008), and to correlate with the level of consciousness. Given the ten Hz sampling rate of MREG
 251 data, we supposed that calculation of its spectral entropy might present an alternative to EEG spectral entropy
 252 analysis by showing similar reductions during sleep (Kumar et al. 2013; Mahon et al. 2008; Vakkuri et al. 2005).
 253 We calculated a single spectral entropy value for whole time series with MATLAB (R2019) using the *pentropy*
 254 function, which produced a spatial map. The method is based on treating the normalized Fast Fourier Transform
 255 (FFT) power spectrum as a probability distribution, which is then used to calculate the Shannon entropy (Shannon
 256 1948). The spectral entropy value describes the irregularity or complexity of the signal, where a noiseless, regular,
 257 and totally predictable signal such as a perfect sine wave has an entropy value of zero, whereas complex signals
 258 with several prominent frequency bands have a higher spectral entropy value. Thus, spectral entropy tends to
 259 decrease when the power in one frequency range starts to dominate.

260 We tested the accuracy of spectral entropy to discriminate sleep from wakefulness. First, we created a model that
 261 included data from only the awake and deepest sleep conditions. As there were 30 fully awake five-minute
 262 segments, we chose for comparison the 30 segments with the deepest sleep state ($88 \pm 12\%$ N1 or N2). We
 263 weighted the sleep states incrementally with increasing depth of sleep (awake=0, N1=1, N2=2, N3=3), multiplied
 264 the weighting values by the corresponding number of 30 second epochs, and calculated the sum to get an EEG-
 265 weighted sleep score for each five-minute segment. Thus, a fully awake five-minute session corresponds to a
 266 minimum EEG weighted sleep score of zero and a five-minute segment of entirely N3 sleep corresponds to a
 267 maximum score of 30. Data were included when sleep states and spectral entropy were both available (A_1 : $n=12$,
 268 A_2 : $n=12$, EC: $n=13$, S_1 : $n=12$, S_2 : $n=12$, S_3 : $n=10$ and S_4 : $n=10$).

269 To test the fitness of spectral entropy to accurately identify sleep, we chose a region of interest (ROI) from visual
 270 cortex (spherical ROI of 5 mm radius, MNI x 12 y -63 z -6) for further analysis, as the fMRI signal in this region
 271 robustly changes across the sleep-wake cycle (Chang et al. 2016; Fukunaga et al. 2006; Liu et al. 2018). We then
 272 compared AASM sleep scores and the ROI spectral entropy results to see whether spectral entropy changes
 273 occurred prior to sleep onset (Figure 3a-b). Next, we calculated the receiver operating curve (ROC) to determine
 274 the separability, sensitivity and specificity of the ROI spectral entropy to distinguish sleep from wakefulness.

275 We then performed a voxel-level analysis to map regions in which spectral entropy distinguished between Awake
 276 versus Sleep states (A_1 versus S_n ; $n=12$ for whom sleep state available). Next, we applied a bandpass filter with

277 *3dTproject* for very low (0.008-0.1 Hz), respiratory (0.11-0.44 Hz), and cardiac (0.52-1.6 Hz) frequencies to enable
 278 a comparison of spectral entropy in relation to these distinct physiological phenomena. The very low range of
 279 0.008-0.1 Hz was chosen to obtain maximum possible coverage of the low frequencies, without cross-talk from
 280 respiratory frequencies. Group mean ranges for respiratory and cardiac frequencies were verified from individual
 281 anesthesia and/or scanner physiological monitoring signals for FFT analysis. As previously shown,
 282 cardiorespiratory distributions in MREG correspond to the frequency ranges in physiological signals (Tuovinen et
 283 al. 2020). First, we defined the peak of the respiratory frequency and then identified, the starting and ending points
 284 of the respiratory power (lowest point around the peak) from the EtCO₂ signals; if not available, we used data from
 285 the respiratory belt of each scan separately. All subjects had some data available (A₁-S₄) but in 7/60 of the datasets
 286 we could not make the definition. Cardiac frequency range was defined similarly from the fingertip peripheral SpO₂
 287 (8/60 missing, but at least one dataset was available for all subjects). Group mean ranges were chosen based on the
 288 lowest and highest values of the individual ranges, with no overlap of other frequency bands.

289 **Power spectral analysis of MREG signal**

290 Using power spectrum analysis, we studied differences between A₁ versus S_n, voxel-wise and for the global signal,
 291 which is known to shift depending on state of arousal (Chang et al. 2016; Liu et al. 2018; Wen and Liu 2016).
 292 Power spectral analysis is an effective tool to separate different sources of brain fluctuations according to different
 293 frequency ranges (Duff et al. 2008). Here, we separated three frequency bands as described in the preceding
 294 section. For each frequency range, we calculated a FFT power density map with the *3dPeriodogram* function in
 295 AFNI for the A₁ and S₁₋₄ scans. The scanning segments together comprised 2861 time points, and FFT was
 296 conducted with 4096 bins such that we ended up with 2048 bins corresponding to 0-5 Hz (0.0024 Hz resolution).
 297 Very low frequency, respiratory, and cardiac verified FFT power frequency ranges were separated from the global
 298 and voxel-wise MREG periodograms using *fslroi* (after specifying the frequency ranges), and the summed power
 299 over each frequency range was calculated using *3dTstat*.

300 **Correlation analysis between spectral entropy and power**

301 Using *fslcc*, spectral entropy difference maps were correlated with the corresponding sum of power maps to
 302 evaluate the extent of their spatial overlap. The three frequency band results of both methods were likewise
 303 correlated to reveal whether the different pulsations involved similar brain regions.

304 **Statistical analysis**

305 We used FSL *randomise* with Threshold-Free Cluster Enhancement and family-wise error (false positive) control
 306 along with a two-sample paired t-test model (A_1 versus S_n , df 11) in the voxel-wise spectral entropy and the FFT
 307 power analyses. We calculated Pearson correlations for sleep state change and spectral entropy ROI in relation to
 308 scan segments (from A_1 to S_2).

309 The ROC curve was calculated to reveal the accuracy of spectral entropy to distinguish sleep and awake states, and
 310 the optimal sensitivity and specificity of the model was calculated (MATLAB R2019). For smart ring activity,
 311 CANTAB data, and head motion data, statistical differences were calculated by a two-sample paired t-test model
 312 using SPSS (IBM SPSS Statistics 26). In the EEG slow oscillation analysis, statistical testing for the difference in
 313 means was made with permutation testing by randomly shuffling the group labels 10,000 times. We transformed
 314 the differences into z-scores, from which statistically significant (two-tailed, $p < 0.05$) results can be obtained.

315 **Results**

316 We studied spectral entropy and power sum of physiological brain pulsations during awake and NREM sleep and
 317 then, investigated their relation to EEG slow oscillation power. We performed main analysis with sample size of 12
 318 or 15 (Figure 1). Data from a representative subject demonstrates the dramatic effect of NREM sleep on the power
 319 intensity of the EEG (Figure 2 b-c) and MREG data in the three extracted pulsation frequency ranges (Figure 2 d-
 320 e). Respiratory and very low frequency power increased sharply and spectral entropy decreased in NREM sleep
 321 (the chosen five-minute segment here includes 80% N2 and 20% N1 sleep), especially when entropy was
 322 calculated in the visual cortex (Figure 2b-e).

323 **Insert Figure 2 about here.**

324 **N1 and N2 states occurred as majority in Sleep scans**

325 Sleep scoring was performed for 15 subjects across the Awake and Sleep scan sessions. Only a single epoch of
 326 sleep was detected for one individual in the Awake eyes open (A_{1-2}) scan sessions. Among the Eyes closed scans,
 327 32 % were scored as NREM sleep, most of which (~88%) were N1. Finally, the Sleep scan sessions (S_{1-4}) included
 328 72-87% N1 or N2 sleep (Table 1, Figure 2a).

To investigate effects of NREM sleep on MREG detected brain pulsations, we selected individual five-minute segments that included the highest amount of sleep. For subject level analysis ($n=12$), the average amount of NREM sleep in the Sleep scan session was 91 ± 11 % (N1: 33 ± 28 %, N2: 57 ± 32 %, N3: 1 ± 3 %, awake: 8 ± 1 %, unknown: 1 ± 3 %), whereas the awake scan (A_1) consisted of wakefulness and only 1 ± 3 % N1 sleep (Table 1).

Insert Table 1 about here.

Frequency ranges for pulsation analysis were determined from physiological EtCO₂ and SpO₂ signals

Group mean ranges for respiratory and cardiac frequencies were obtained from individual physiological monitoring EtCO₂ or respiratory belt and SpO₂ signal data based on the minimum and maximum points of the highest peak. The peaking value was significantly lower for EtCO₂ in S_1 ($p=0.012$) and S_2 ($p=0.029$) when compared to EO and for SpO₂ in EC ($p=0.0009$) and S_2 ($p=0.001$), all when compared to EO.

Insert Table 2 about here.

Smart ring corroborated sleep deprivation and CANTAB revealed slower reaction time after sleep deprivation

Based on pilot data for the successful measurement of synchronous multimodal MREG and DC-EEG signal (Korhonen et al. 2014), we recruited healthy subjects for a sleep monitoring study, and requested them to use the ring for at least 24 hours preceding the Awake scan session likewise to verify sleep deprivation preceding the Sleep scan session. Based on the smart ring data, we observed only 9 ± 19 minutes of sleep before Sleep scan sessions, corroborating that our subjects were indeed sleep deprived at that time (14 of 15 subjects). Three/15 subjects had incomplete smart ring data during the night of sleep deprivation (16, 25 and 57 minutes) and nine/15 subjects had some missing data during the entire 24 hours before the scan. After sleep deprivation, the subjects exhibited significantly increased reaction times, indicative of reduced vigilance compared to their results during the Awake scan session following a normal night's sleep (CANTAB reaction time test: $429 \pm 29 > 406 \pm 30$ ms, $p=0.028$).

Full band spectral entropy of MREG decreased during NREM sleep

We focused our analysis of MREG spectral entropy in a ROI placed in the visual cortex (Figure 3), a brain area known to show changes in very low frequency pulsations during periods of decreased vigilance and sleep (Chang et

al. 2016; Fukunaga et al. 2006; Liu et al. 2018). Thus, we evaluated whether MREG spectral entropy results in visual cortex can distinguish Awake and Sleep segments. As expected, the weighted sleep staging data (awake, N1, N2, N3) showed linear relationships with the scan segments (A_1 - S_2) ($r = -0.952$, $p < 0.01$), closely resembling the association observed between the MREG spectral entropy ROI data and the scan segments ($r = -0.956$, $p < 0.01$) (Figure 3a-b, d). The accuracy whereby spectral entropy in the visual cortex ROI separated sleep from wakefulness was further investigated using receiver operating curve (ROC) analysis. By comparing Awake segments ($n = 30$, fully awake) against Sleep ($n = 30$, $88 \pm 12\%$ NREM sleep), we were able to separate sleep from wakefulness with high accuracy (Area Under the Curve AUC=0.88, $p < 0.0001$, Figure 3f), with a sensitivity of 93 % and specificity of 77 %. These results show that spectral entropy, which is a marker of vigilance, indeed reduced with increasing NREM sleep depth.

To analyze spatial differences in entropy between Awake and Sleep, we calculated full band voxel-wise maps in the Eyes open (A_{1-2}) and Sleep segments (S_{1-4}). We chose the A_1 segment for the analysis due to having the highest awake state (99% awake, and highest vigilance based on spectral entropy ROI). We compared the Awake data versus the time series with the highest proportion of NREM sleep ($91 \pm 12\%$ NREM sleep, A_1 versus S_n , $df = 11$). The occipital, parietal and temporal lobes and cerebellum exhibited significant reductions in spectral entropy ($p < 0.05$, $df = 11$, Figure 3c) during NREM sleep, whereas parts of the frontal lobe did not show spectral entropy changes.

Insert Figure 3 about here.

Global MREG signal revealed enhanced whole-brain pulsatility

Next, we investigated whether the reduction in spectral entropy during NREM sleep might be linked to an increase in brain pulsation strength. FFT power of the whole-brain MREG signal was calculated to assess the strength of each pulsation. As global fMRI is known to reflect arousal fluctuations (Chang et al. 2016; Liu et al. 2018; Wen and Liu 2016), we first calculated the summed power for the global MREG spectrum. As hypothesized, the spectral power increased markedly in the very low and respiratory frequencies, and to a lesser extent in the cardiac pulsation range (Figure 4). Taken together, the power in each of the three physiological frequency bands revealed significant increases during NREM sleep compared to waking, suggesting that physiological brain pulsations are globally enhanced during sleep.

382 **Insert Figure 4 about here.**

383 **Sleep induced entropy and power changes occurred mainly in very low and respiratory pulsations**

384 To investigate the spatial distribution of brain pulsations, we compared spectral power and spectral entropy maps
 385 between Awake and Sleep states, which showed significant differences mainly in very low and respiratory
 386 frequency ranges. As the power increased in very low, respiratory, and cardiac frequency bands in the transition to
 387 sleep, spectral entropy decreased in very low and respiratory bands. Figure 5 shows spectral entropy and power
 388 changes across all brain voxels, for evaluating the relative contributions of the three physiological brain pulsations
 389 during Awake and Sleep conditions.

390 The largest state-dependent change in power and spectral entropy occurred in the very low frequency band
 391 extending over posterior parts of the brain. The correlation coefficient between power and spectral entropy
 392 differences in Awake versus Sleep ($r=0.45$) indicated widespread overlapping regions, especially in posterior parts
 393 of the brain. There was a power increase with no decrease in entropy in the brain stem and ventricles, and
 394 unchanged power along with low entropy values in frontal cortex. Thus, entropy can change without concomitant
 395 power changes.

396

397 The respiratory pulsation power increased and its spectral entropy decreased significantly during sleep ($p < 0.05$, df
 398 11, Figures 4-5). The power increase was located in upper posterior brain areas and in the ventricles, overlapping
 399 with the regions showing a very low frequency increase ($r=0.34$, Figures 4-5). The most significant power increase
 400 occurred in typical Awake activation areas (Downar et al. 2000), thus involving the primary sensorimotor and
 401 auditory cortices, along with the V1-V2 visual areas. Power also increased in the CSF filled spaces of the lateral
 402 and central sulci, indicating strong respiratory pulsatility in sleep. In contrast to the increased respiratory frequency
 403 power, the decreased respiratory frequency spectral entropy was located more towards the basal brain structures
 404 and in the frontal pole, with less overlap with the power changes seen in upper posterior regions although still
 405 showing some overlap in visual and auditory cortices ($r=0.2$). Spectral entropy changes were found sub-cortically
 406 near the gray matter/white matter border and more prominently in white matter, which has greater venous than
 407 arterial circulation. There was decreased spectral entropy in the brain stem, which did not overlap with power
 408 changes. Overall, very low and respiratory frequency power bands showed widespread increase during NREM
 409 sleep, in conjunction with decreased entropy in partially overlapping brain areas.

410 The power of cardiac pulsation increased in somatosensory cortex during sleep ($p < 0.05$, df 11, Figure 5b, bottom),
 411 but spectral entropy did not change in the cardiac frequency range. Overall, we found that power increased in
 412 widespread brain regions for all three frequency ranges, whereas spectral entropy decreased in very low and
 413 respiratory frequency bands during NREM sleep.

414 **Insert Figure 5 about here.**

415 **EEG slow oscillation changes overlapped with very low, respiratory and cardiac pulsation changes of**
 416 **MREG**

417 Next, we studied whether pulsation power increase and spectral entropy decrease are associated with the SWA
 418 (0.5-4 Hz) power and slow oscillation (0.2-2 Hz) power of EEG, as delta power changes have previously been
 419 connected with BOLD signal and CSF flow (Fultz et al. 2019) and with increased beta-amyloid clearance (Hablitz
 420 et al. 2019). The sleep induced EEG-derived slow oscillation sleep changes were located in the right midline
 421 parietal and occipital areas (Figure 6). These changes spatially overlapped with regions of increased power of all
 422 physiological brain pulsations having reduced spectral entropy. The highest overall overlap was seen in EEG slow
 423 oscillation frequency and the MREG respiratory frequency range. We did not find significant changes in SWA
 424 (0.5-4 Hz) power.

425 **Insert Figure 6 about here.**

426 **Discussion**

427 We studied spectral entropy and power sum changes in MREG data in very low, respiratory, and cardiac frequency
 428 bands during NREM sleep and while awake, and examined spatial similarities with concomitant EEG slow
 429 oscillation power changes. To our knowledge, this is the first study to report brain-wide enhancement and reduced
 430 spectral entropy in very low and respiratory frequency pulsations, and a lesser extent, increased cardiac frequency
 431 power. We demonstrate that regional neurophysiological changes in EEG slow oscillation power specifically co-
 432 localize with the same regions as MREG changes for all three physiological pulsations. The interaction between
 433 0.05 Hz CSF pulsations with neural and hemodynamic oscillations has been shown to increase during human sleep
 434 (Fultz et al. 2019), which is supported by our present observation of these changes in mainly N2 sleep. Together

with previous literature, our new observations suggest that the hydrodynamic properties governing CSF movement in the brain are regulated by physiological pulsations across the sleep-wake cycle.

Vasomotor pulsations dominate during NREM sleep

Present results are in accord with earlier literature on the conventional BOLD signal, which showed increased very low frequency fluctuations in posterior brain regions during light sleep and episodes of low waking vigilance (Chang et al. 2016; Fukunaga et al. 2006; Horovitz et al. 2008; Liu et al. 2018). Moreover, slow deactivation cycles of the cholinergic neurons of the nucleus basalis of Meynert have been shown to precede widely distributed hemodynamic BOLD signal increase in posterior brain regions (Liu et al. 2018). Present results show increases in very low frequency signals in posterior brain regions virtually identical to those reported by Liu et al. Spectral entropy of the EEG signal has been shown to decrease with sleep depth, where the frequency range moves towards lower frequencies and obtains a nadir during deepest sleep (Mahon et al. 2008). We found a similar pattern in MREG recordings, notably in the cerebral cortex. Therefore, we propose that the mechanism underlying the enhanced and stabilized very low frequency signal must start to dominate in the MREG 0-5 Hz frequency range.

Low frequency BOLD fluctuations are widely attributed to functionally connected hemodynamic oscillations that are tightly coupled to neuronal activity during wakefulness (Hutchison et al. 2013; Korhonen et al. 2014). Part of this low frequency activity at ≤ 0.1 Hz has been ascribed a vascular origin arising from vasomotor waves or slow sinusoidal hemodynamic oscillations (Biswal et al. 1995; Kiviniemi et al. 2000; Kiviniemi et al. 2016; Rayshubskiy et al. 2014; Wang et al. 2008). As, similarly shown during N2 sleep by Özbay et al. peripheral vasomotor constrictions driven by central K-complexes precede the global BOLD signal by some 11 seconds in human brain (Özbay et al. 2019). Vasomotor waves are slow, seemingly spontaneous undulations in the arteriolar wall tension that control vessel wall pulsatility and local flow resistance, which consequently influence perfusion in downstream vascular territories (Preiss and Polosa 1974). The force of the driving pulsation depends on various factors, including the circulatory perfusion pressure and elasticity of the vessel wall, both of which are regulated by smooth muscle vasomotor tonus in the arterial wall (Hadaczek et al. 2006). Recent data have indicated that vasomotor waves can move injected tracers along the perivascular space, thus serving as a glymphatic driver (van Veluw et al. 2020).

Hillmann and colleagues have detected two distinct very low frequency phenomena using wide-field optical imaging in mice during mice (Ma et al. 2016). Urethane-anesthesia produces similar delta power activity as ketamine/xylazine anesthesia (Chauvette et al. 2011), which has been linked to increased glymphatic influx (Hablitz et al. 2019) of a similar magnitude as that occurring during natural sleep (Xie et al. 2013). A 0.04 Hz fluctuation present only in anesthesia data overshadowed the faster hemodynamic changes coupled to spontaneous neuronal activity (Ma et al. 2016), and only upon removing the 0.04 Hz vasomotor fluctuation, did the underlying neuronal activity-coupled and functionally connected hemodynamic signal emerge. Interestingly, we also found a peak power value at 0.04 Hz in the global MREG (Figure 4), which might arise from a similar source. Emergence of this new peaking frequency range during sleep likely decreased the spectral entropy. Plausibly, the very low frequency BOLD signal might originate from non-neuronal sources such as vasomotor activity, especially during sleep.

Respiratory power increases and spectral entropy decreases during NREM sleep

For the first time, we present evidence that sleep specific brain pulsations change in rhythm with the respiratory frequency, with increased power and reduced complexity. Although physiological pulsations can affect the BOLD signal, this interaction has not been widely studied due to technical temporal limitations of conventional fMRI (Huotari et al. 2019; Kiviniemi et al. 2016). The present acquisition of fast MREG data enable a robust separation of the cardiac and respiratory pulsations due to absence of signal aliasing over the very low frequencies (Huotari et al. 2019; Kiviniemi et al. 2016; Raitamaa et al. 2018).

During inspiration, the reduced intrathoracic pressure induces outflow of venous blood from brain that is counterbalanced by an inward movement of CSF, which is a necessary consequence of the confinement of brain within the incompressible dural venous sinuses and intracranial space (Dreha-Kulaczewski et al. 2015; Klose et al. 2000; Vinje et al. 2019; Yamada et al. 2013). This mechanism is bound also to change the perivenous CSF space, which, together with counter-phase venous blood volume changes, creates a perivenous CSF pump. In addition, pulmonary ventilation and upper airway resistance increase during sleep (Sowho et al. 2014; Trinder et al. 1992; Wiegand et al. 1989; Worsnop et al. 1998). The increased intrathoracic ventilation pressures may consequently promote the (peri)venous CSF pumping action in the brain, manifesting in increased respiratory frequency power in the MREG signal (Dreha-Kulaczewski et al. 2015; Matsumae et al. 2019; Vinje et al. 2019). In addition, our results showing decreased spectral entropy in the respiratory frequency range during N1-2 sleep are in accord with

findings that the respiratory pattern is more regular during NREM sleep compared with waking (Malik et al. 2012). The increased and stabilized physiological pulsations during sleep would likely increase CSF flow towards the neuropil, which might inflate the extracellular space, as has been shown to occur during sleep/wake transitions (Xie et al. 2013).

Furthermore, the increased respiratory and very low frequency brain pulsations in NREM sleep overlap in posterior brain regions, which may be mediated by shared autonomic pressure control mechanisms (Özbay et al. 2019). In line with previous literature (Ma et al. 2016; Özbay et al. 2019), we suppose that the physiological pulsations may overpower neurovascularly coupled activity in the primary sensory processing areas during mainly N2 sleep. Thus, the enhanced brain clearance in light N2 sleep may arise in cortical areas that process sensory information in the awake state (Downar et al. 2000).

Cardiovascular pulsations enhance in somatosensory cortex

Microscopic studies in rodent brain have shown that parenchymal cardiovascular pulsatility is the main contributor to CSF convection (Iliff et al. 2013; Mestre et al. 2018), whereas our whole brain analysis results do not show large scale changes during NREM sleep. Cardiovascular pulsatility provides energy substrates supporting neuronal activity, which overall stays stable. However, we saw increased cardiac power in somatosensory cortex, a region that is known to remain active during sleep (Ramot et al. 2013). Lecci et al. found that a 0.02 Hz oscillation occurred in somatosensory cortex in NREM sleep in mice and humans, and went on to suggest that such oscillations are mainly caused by sleep spindles tightly coupled with heart rate (Lecci et al. 2017). While the present data suggest that vasomotor and respiratory pulsations dominate throughout the neuropil during sleep, the interactions between the physiological pulsations need to be further investigated.

Coupling between EEG and MREG pulsations

Recent studies have shown that the delta power changes in EEG are tightly related to BOLD activity changes along with faster CSF flow (Fultz et al. 2019), and that higher influx of CSF in the brain parenchyma allows increased solute transport from the brain (Hablitz et al. 2019). Fultz et al. showed that the onset of slow-delta EEG occurred 6.4 seconds prior to the CSF peak, and concluded that such coupling was strongest during sleep (Fultz et al. 2019). In addition, they found that BOLD and CSF signals were anticorrelated in the cortical grey matter, thus suggesting an alternation between BOLD and CSF flow, in accord with the constraint of constant intracranial volume.

516 Interestingly, recent work showed that controlled deep inspirations during wakefulness were followed by fMRI and
 517 CSF changes similar to those occurring during NREM 1 and 2 sleep, thus suggesting that respiration is actually a
 518 driver for EEG and fMRI signal (Picchioni et al. 2021). Along the same lines, an increased respiratory effect on
 519 neuronal brain activity has been detected in intracranial needle measurements and MREG studies of epileptic
 520 patients (Herrero et al. 2018; Kananen et al. 2020; Kananen et al. 2018; Zelano et al. 2016). Our findings suggest
 521 that the main changes in EEG occurred in slow oscillation power, which increased in concert with higher pulsation
 522 strength and reduced spectral entropy. Mechanistically, slow oscillation power increases along with elevated
 523 interstitial electrolyte levels and narrowing of the intercellular space (Ding et al. 2016; Hablitz et al. 2019; Xie et al.
 524 2013); such mechanisms are likely to also occur during increased SWA human sleep. Since pulsation changes
 525 appeared in early sleep (NREM 1-2) and overlapped with EEG slow oscillation changes, we argue that these
 526 findings could reflect a direct physiological interaction between electrohydrodynamic pulsations rather than arising
 527 from an interaction with the faster neurophysiological pulsations that markedly decline in power during sleep.

528 **Limitations**

529 The Awake scan was performed at 4-6 PM and the Sleep scan at 6-8 AM, such that circadian rhythms might have
 530 added a confound to our results. To minimize that possibility, we selected for analysis those segments that included
 531 the highest amount of sleep according to AASM sleep scoring criteria. In terms of naturalistic sleep, the fMRI
 532 laboratory is a challenging environment for attaining deep sleep; unfortunately, we only observed one epoch of N3
 533 sleep. We conducted the sleep deprivation prior to the Sleep scan session to help subjects fall asleep in the scanner.
 534 While somewhat effective, this intervention may have increased sympathetic activity as is described to occur after
 535 sleep deprivation (Tobaldini et al. 2017). The sleep deprivation took place at home instead of the laboratory
 536 environment, such that we are reliant upon surrogate measures of sleep deprivation in some subjects.

537
 538 We did not perform screening for sleep apnea or other sleep disorders since all included subjects were young and
 539 generally healthy. Our sample size was small (n=12-15) calling for replication of some analysis in additional
 540 subjects. Subjective sleep quality was not assessed with validated questionnaires which we consider to be another
 541 limitation. There was no *a priori* statistical power analysis. We had no criteria for the eating schedule for the
 542 subjects. Further work is required to investigate the exact source of different physiological changes over the whole
 543 0.008-5 Hz frequency band that is accessible with direct current EEG and MREG.

Conclusions

NREM sleep alters all three physiological brain pulsations, with the greatest power increases and spectral entropy decreases in the vasomotor and respiratory pulsations. In addition, we found small power increase in cardiac frequency band. Electrophysiological slow oscillation power increased in brain regions overlapping with all pulsation changes, suggesting that these pulsations participate in sleep-related electrophysiological brain processes with an inverse relation to pulsation frequency. This inverse frequency change robustly reduces entropy of the brain physiological signal spectrum, enabling a clear dissociation of sleep and awake states from MREG data. We suggest the physiological brain pulsations alongside slow wave electrophysiological changes in NREM sleep likely contribute to elevated CSF flow.

References

- Allen PJ, Josephs O, Turner R (2000) A method for removing imaging artifact from continuous EEG recorded during functional MRI. *Neuroimage* 12:230-239.
- Allen PJ, Polizzi G, Krakow K, Fish DR, 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.
- Assländer J, Zahneisen B, Hugger T, Reisert M, Lee H-, LeVan P, Hennig J (2013) Single shot whole brain imaging using spherical stack of spirals trajectories. *Neuroimage* 73:59-70.
- Berger H (1901) Zur Lehre von der Blutzirkulation in der Schädelhöhle des Menschen namentlich unter dem Einfluss von Medikamenten. Jena: Verlag von Gustav Fischer, .
- Biswal B, Zerrin Yetkin F, Haughton VM, Hyde JS (1995) Functional connectivity in the motor cortex of resting human brain using echo-planar mri. *Magn Reson Med* 34:537-541.
- Brancaccio A, Tabarelli D, Bigica M, Baldauf D (2020) Cortical source localization of sleep-stage specific oscillatory activity. *Sci Rep* 10:6976.
- CANTAB® (2019) [Cognitive assessment software]. Cambridge Cognition. All rights reserved. www.cantab.com.

- 574 Chang C, Leopold DA, Schölvinck ML, Mandelkow H, Picchioni D, Liu X, Ye FQ, Turchi JN, Duyn JH (2016)
575 Tracking brain arousal fluctuations with fMRI. *Proc Natl Acad Sci U S A* 113:4518-4523.
- 576 Chauvette S, Crochet S, Volgushev M, Timofeev I (2011) Properties of slow oscillation during slow-wave sleep
577 and anesthesia in cats. *J Neurosci* 31:14998-15008.
- 578 Cox RW (1996) AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages.
579 *Comput Biomed Res* 29:162-173.
- 580 de Zambotti M, Rosas L, Colrain IM, Baker FC (2017) The Sleep of the Ring: Comparison of the OURA Sleep
581 Tracker Against Polysomnography. *Behav Sleep Med* 17:1-15.
- 582 Ding F, O'donnell J, Xu Q, Kang N, Goldman N, Nedergaard M (2016) Changes in the composition of brain
583 interstitial ions control the sleep-wake cycle. *Sci* 352:550-555.
- 584 Downar J, Crawley AP, Mikulis DJ, Davis KD (2000) A multimodal cortical network for the detection of changes
585 in the sensory environment. *Nat Neurosci* 3:277-283.
- 586 Dreha-Kulaczewski S, Joseph AA, Merboldt K-, Ludwig H-, Gärtner J, Frahm J (2015) Inspiration is the major
587 regulator of human CSF flow. *J Neurosci* 35:2485-2491.
- 588 Duff EP, Johnston LA, Xiong J, Fox PT, Mareels I, Egan GF (2008) The power of spectral density analysis for
589 mapping endogenous BOLD signal fluctuations. *Hum Brain Mapp* 29:778-790.
- 590 Fukunaga M, Horovitz SG, van Gelderen P, de Zwart JA, Jansma JM, Ikonomidou VN, Chu R, Deckers RHR,
591 Leopold DA, Duyn JH (2006) Large-amplitude, spatially correlated fluctuations in BOLD fMRI signals during
592 extended rest and early sleep stages. *Magn Reson Imaging* 24:979-992.
- 593 Fultz NE, Bonmassar G, Setsompop K, Stickgold RA, Rosen BR, Polimeni JR, Lewis LD (2019) Coupled
594 electrophysiological, hemodynamic, and cerebrospinal fluid oscillations in human sleep. *Sci* 366:628-631.
- 595 Hablitz LM, Vinitsky HS, Sun Q, Staeger FF, Sigurdsson B, Mortensen KN, Lilius TO, Nedergaard M (2019)
596 Increased glymphatic influx is correlated with high EEG delta power and low heart rate in mice under anesthesia.
597 *Sci Adv* 5:2.
- 598 Hadaczek P, Yamashita Y, Mirek H, Tamas L, Bohn MC, Noble C, Park JW, Bankiewicz K (2006) The
599 "Perivascular Pump" Driven by Arterial Pulsation Is a Powerful Mechanism for the Distribution of Therapeutic
600 Molecules within the Brain. *Mol Ther* 14:69-78.
- 601 Herrero JL, Khuvis S, Yeagle E, Cerf M, Mehta AD (2018) Breathing above the brain stem: Volitional control and
602 attentional modulation in humans. *J Neurophysiol* 119:145-159.
- 603 Horovitz SG, Braun AR, Carr WS, Picchioni D, Balkin TJ, Fukunaga M, Duyn JH (2009) Decoupling of the brain's
604 default mode network during deep sleep. *Proc Natl Acad Sci U S A* 106:11376-11381.
- 605 Horovitz SG, Fukunaga M, De Zwart JA, Van Gelderen P, Fulton SC, Balkin TJ, Duyn JH (2008) Low frequency
606 BOLD fluctuations during resting wakefulness and light sleep: A simultaneous EEG-fMRI study. *Hum Brain Mapp*
607 29:671-682.
- 608 Hugger T, Zahneisen B, LeVan P, Lee KJ, Lee H-, Zaitsev M, Hennig J (2011) Fast undersampled functional
609 magnetic resonance imaging using nonlinear regularized parallel image reconstruction. *PLoS ONE* 6:12.
- 610 Huotari N, Raitamaa L, Helakari H, Kananen J, Raatikainen V, Rasila A, Tuovinen T, Kantola J, Borchardt V,
611 Kiviniemi VJ, Korhonen VO (2019) Sampling rate effects on resting state fMRI metrics. *Front Neurosci* 13:279.

- Hutchison RM, Womelsdorf T, Allen EA, Bandettini PA, Calhoun VD, Corbetta M, Della Penna S, Duyn JH, Glover GH, Gonzalez-Castillo J, Handwerker DA, Keilholz S, Kiviniemi V, Leopold DA, de Pasquale F, Sporns O, Walter M, Chang C (2013) Dynamic functional connectivity: Promise, issues, and interpretations. *Neuroimage* 80:360-378.
- Iliff JJ, Wang M, Zeppenfeld DM, Venkataraman A, Plog BA, Liao Y, Deane R, Nedergaard M (2013) Cerebral arterial pulsation drives paravascular CSF-Interstitial fluid exchange in the murine brain. *J Neurosci* 33:18190-18199.
- Jenkinson M, Beckmann CF, Behrens TEJ, Woolrich MW, Smith SM (2012) FSL. *Neuroimage* 62:782-790.
- Jenkinson M, Bannister P, Brady M, Smith S (2002) Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage* 17:825-841.
- Kananen J, Helakari H, Korhonen V, Huotari N, Järvelä M, Raitamaa L, Raatikainen V, Rajna Z, Tuovinen T, Nedergaard M, Jacobs J, LeVan P, Ansakorpi H, Kiviniemi V (2020) Respiratory-related brain pulsations are increased in epilepsy—a two-centre functional MRI study. *Brain Commun* 2:2.
- Kananen J, Tuovinen T, Ansakorpi H, Rytty S, Helakari H, Huotari N, Raitamaa L, Raatikainen V, Rasila A, Borchardt V, Korhonen V, LeVan P, Nedergaard M, Kiviniemi V (2018) Altered physiological brain variation in drug-resistant epilepsy. *Brain Behav* 8:9.
- Kaufmann C, Wehrle R, Wetter TC, Holsboer F, Auer DP, Pollmächer T, Czisch M (2006) Brain activation and hypothalamic functional connectivity during human non-rapid eye movement sleep: An EEG/fMRI study. *Brain* 129:655-667.
- Kiviniemi V, Wang X, Korhonen V, Keinänen T, Tuovinen T, Autio J, Levan P, Keilholz S, Zang Y-, Hennig J, Nedergaard M (2016) Ultra-fast magnetic resonance encephalography of physiological brain activity-Glymphatic pulsation mechanisms?. *J Cereb Blood Flow Metab* 36:1033-1045.
- Kiviniemi V, Jauhainen J, Tervonen O, Pääkkö E, Oikarinen J, Vainionpää V, Rantala H, Biswal B (2000) Slow vasomotor fluctuation in fMRI of anesthetized child brain. *Magn Reson Med* 44:373-378.
- Klose U, Strik C, Kiefer C, Grodd W (2000) Detection of a relation between respiration and CSF pulsation with an echoplanar technique. *J Magn Reson Imaging* 11:438-444.
- Korhonen V, Hiltunen T, Myllylä T, Wang X, Kantola J, Nikkinen J, Zang Y-, LeVan P, Kiviniemi V (2014) Synchronous multiscale neuroimaging environment for critically sampled physiological analysis of brain function: hepta-scan concept. *Brain Connect* 4:677-689.
- Kumar R, Ramaswamy R, Nath Mallick B (2013) Local Properties of Vigilance States: EMD Analysis of EEG Signals during Sleep-Waking States of Freely Moving Rats. *PLoS ONE* 8:10.
- Lecci S, Fernandez LMJ, Weber FD, Cardis R, Chatton J-, Born J, Lüthi A (2017) Coordinated infraslow neural and cardiac oscillations mark fragility and offline periods in mammalian sleep. *Sci Adv* 3:2.
- Lee H-, Zahneisen B, Hugger T, LeVan P, Hennig J (2013) Tracking dynamic resting-state networks at higher frequencies using MR-encephalography. *Neuroimage* 65:216-222.
- Liu X, De Zwart JA, Schölvinck ML, Chang C, Ye FQ, Leopold DA, Duyn JH (2018) Subcortical evidence for a contribution of arousal to fMRI studies of brain activity. *Nat Commun* 9, 395.
- Ma Y, Shaik MA, Kozberg MG, Kim SH, Portes JP, Timerman D, Hillman EMC (2016) Resting-state hemodynamics are spatiotemporally coupled to synchronized and symmetric neural activity in excitatory neurons. *Proc Natl Acad Sci U S A* 113:E8463-E8471.

- 652 Mahon P, Greene BR, Lynch EM, McNamara B, Shorten GD (2008) Can state or response entropy be used as a
653 measure of sleep depth? *Anaesthesia* 63:1309-1313.
- 654 Malik V, Smith D, Lee-Chiong Jr. T (2012) Respiratory physiology during sleep . *Sleep Med Clin* 7:497-505.
- 655 Mason JW, Ramseth DJ, Chanter DO, Moon TE, Goodman DB, Mendzelevski B (2007) Electrocardiographic
656 reference ranges derived from 79,743 ambulatory subjects. *J Electrocardiol* 40:228-234.e8.
- 657 Matsumae M, Kuroda K, Yatsushiro S, Hirayama A, Hayashi N, Takizawa K, Atsumi H, Sorimachi T (2019)
658 Changing the currently held concept of cerebrospinal fluid dynamics based on shared findings of cerebrospinal
659 fluid motion in the cranial cavity using various types of magnetic resonance imaging techniques. *Neurol Med -Chir*
660 59:133-146.
- 661 Mehrabadi MA, Azimi I, Sarhaddi F, Axelin A, Niela-Vilén H, Myllyntausta S, Stenholm S, Dutt N, Liljeberg P,
662 Rahmani AM (2020) Sleep tracking of a commercially available smart ring and smartwatch against medical-grade
663 actigraphy in everyday settings: Instrument validation study. *JMIR mHealth uHealth* 8:10.
- 664 Mestre H, Tithof J, Du T, Song W, Peng W, Sweeney AM, Olveda G, Thomas JH, Nedergaard M, Kelley DH
665 (2018) Flow of cerebrospinal fluid is driven by arterial pulsations and is reduced in hypertension. *Nat Commun*
666 9:4878.
- 667 Özbay PS, Chang C, Picchioni D, Mandelkow H, Chappel-Farley MG, van Gelderen P, de Zwart JA, Duyn J
668 (2019) Sympathetic activity contributes to the fMRI signal. *Commun Biolog* 2:1.
- 669 Penzel T, Kantelhardt JW, Lo C-, Voigt K, Vogelmeier C (2003) Dynamics of heart rate and sleep stages in
670 normals and patients with sleep apnea. *Neuropsychopharmacology* 28:S48-S53.
- 671 Picchioni D, Özbay PS, Mandelkow H, Zwart JA, Wang Y, Gelderen P, Duyn J (2021) Autonomic arousals
672 contribute to brain fluid pulsations during sleep. *BioRxiv* doi: <https://doi.org/10.1101/2021.05.04.442672>.
- 673 Posse S, Ackley E, Mutihac R, Zhang T, Hummatov R, Akhtari M, Chohan M, Fisch B, Yonas H (2013) High-
674 speed real-time resting-state fMRI using multi-slab echo-volumar imaging. *Front Human Neurosci* 7:479.
- 675 Preiss G, Polosa C (1974) Patterns of sympathetic neuron activity associated with Mayer waves. *Am J Physiol*
676 226:724-730.
- 677 Raitamaa L, Korhonen V, Huotari N, Raatikainen V, Hautaniemi T, Kananen J, Rasila A, Helakari H, Zienkiewicz
678 A, Myllylä T, Borchardt V, Kiviniemi V (2018) Breath hold effect on cardiovascular brain pulsations – A
679 multimodal magnetic resonance encephalography study. *J Cereb Blood Flow Metab* 39:2471-2485.
- 680 Ramot M, Fisch L, Davidesco I, Harel M, Kipervasser S, Andelman F, Neufeld MY, Kramer U, Fried I, Malach R
681 (2013) Emergence of sensory patterns during sleep highlights differential dynamics of REM and non-REM sleep
682 stages. *J Neurosci* 33:14715-14728.
- 683 Rayshubskiy A, Wojtasiewicz TJ, Mikell CB, Bouchard MB, Timerman D, Youngerman BE, McGovern RA, Otten
684 ML, Canoll P, McKhann GM, Hillman EMC (2014) Direct, intraoperative observation of ~0.1Hz hemodynamic
685 oscillations in awake human cortex: Implications for fMRI. *Neuroimage* 87:323-331.
- 686 Russo MA, Santarelli DM, O'Rourke D (2017) The physiological effects of slow breathing in the healthy
687 human. *Breathe* 13:298-309.
- 688 Shannon CE (1948) The Mathematical Theory of Communication. *Bell System Technical Journal* 27:379–423,
689 623–656.
- 690 Silvani A, Bojic T, Franzini C, Lenzi P, Walker AM, Grant DA, Wild J, Zoccoli G (2004) Sleep-related changes in
691 the regulation of cerebral blood flow in newborn lambs. *Sleep* 27:36-41.

- 692 Sloots JJ, Biessels GJ, Zwanenburg JJM (2020) Cardiac and respiration-induced brain deformations in humans
693 quantified with high-field MRI. *Neuroimage* 210:116581.
- 694 Smith SM (2002) Fast robust automated brain extraction. *Hum Brain Mapp* 17:143-155.
- 695 Sowho M, Amatoury J, Kirkness JP, Patil SP (2014) Sleep and respiratory physiology in adults. *Clin Chest Med*
696 35:469-481.
- 697 Tait MJ, Saadoun S, Bell BA, Papadopoulos MC (2008) Water movements in the brain: role of aquaporins. *Trends*
698 *Neurosci* 31:37-43.
- 699 Tobaldini E, Costantino G, Solbiati M, Cogliati C, Kara T, Nobili L, Montano N (2017) Sleep, sleep deprivation,
700 autonomic nervous system and cardiovascular diseases. *Neurosci Biobehav Rev* 74:321-329.
- 701 Trinder J, Whitworth F, Kay A, Wilkin P (1992) Respiratory instability during sleep onset. *J Appl Physiol* 73:2462-
702 2469.
- 703 Tuovinen T, Kananen J, Rajna Z, Lieslehto J, Korhonen V, Rytty R, Mattila H, Huotari N, Raitamaa L, Helakari H,
704 Elseoud AA, Krüger J, LeVan P, Tervonen O, Hennig J, Remes AM, Nedergaard M, Kiviniemi V (2020) The
705 variability of functional MRI brain signal increases in Alzheimer's disease at cardiorespiratory frequencies. *Sci Rep*
706 10:1.
- 707 Vakkuri A, Yli-Hankala A, Sandin R, Mustola S, Høymork S, Nyblom S, Talja P, Sampson T, Van Gils M,
708 Viertiö-Oja H (2005) Spectral entropy monitoring is associated with reduced propofol use and faster emergence in
709 propofol-nitrous oxide-alfentanil anesthesia. *Anesthesiology* 103:274-279.
- 710 van Veluw SJ, Hou SS, Calvo-Rodriguez M, Arbel-Ornath M, Snyder AC, Frosch MP, Greenberg SM, Bacsikai BJ
711 (2020) Vasomotion as a Driving Force for Paravascular Clearance in the Awake Mouse Brain. *Neuron* 105:549-
712 561.
- 713 Viertiö-Oja H, Maja V, Särkelä M, Talja P, Tenkanen N, Tolvanen-Laakso H, Paloheimo M, Vakkuri A, Yli-
714 Hankala A, Meriläinen P (2004) Description of the Entropy™ algorithm as applied in the Datex-Ohmeda 5/5™
715 Entropy Module. *Acta Anaesthesiol Scand* 48:154-161.
- 716 Vinje V, Ringstad G, Lindstrøm EK, Valnes LM, Rognes ME, Eide PK, Mardal K- (2019) Respiratory influence on
717 cerebrospinal fluid flow – a computational study based on long-term intracranial pressure measurements. *Sci Rep*
718 9:1.
- 719 Wang HH, Menezes NM, Ming WZ, Ay H, Koroshetz WJ, Aronen HJ, Karonen JO, Liu Y, Nuutinen J, Wald LL,
720 Sorensen AG (2008) Physiological noise in MR images: An indicator of the tissue response to ischemia?. *J Magn*
721 *Reson Imaging* 27:866-871.
- 722 Wen H, Liu Z (2016) Broadband electrophysiological dynamics contribute to global resting-state fMRI signal. *J*
723 *Neurosci* 36:6030-6040.
- 724 Wiegand L, Zwillich CW, White DP (1989) Collapsibility of the human upper airway during normal sleep. *J Appl*
725 *Physiol* 66:1800-1808.
- 726 Wong CW, DeYoung PN, Liu TT (2016) Differences in the resting-state fMRI global signal amplitude between the
727 eyes open and eyes closed states are related to changes in EEG vigilance. *Neuroimage* 124:24-31.
- 728 Worsnop C, Kay A, Pierce R, Kim Y, Trinder J (1998) Activity of respiratory pump and upper airway muscles
729 during sleep onset. *J Appl Physiol* 85:908-920.
- 730 Xie L, Kang H, Xu Q, Chen MJ, Liao Y, Thiagarajan M, O'Donnell J, Christensen DJ, Nicholson C, Iliff JJ,
731 Takano T, Deane R, Nedergaard M (2013) Sleep drives metabolite clearance from the adult brain. *Sci* 342:373-377.

- 732 Yamada S, Miyazaki M, Yamashita Y, Ouyang C, Yui M, Nakahashi M, Shimizu S, Aoki I, Morohoshi Y,
 733 McComb JG (2013) Influence of respiration on cerebrospinal fluid movement using magnetic resonance spin
 734 labeling. *Fluids Barriers CNS* 10:1.
- 735 Zahneisen B, Hugger T, Lee KJ, Levan P, Reisert M, Lee H-, Assländer J, Zaitsev M, Hennig J (2012) Single shot
 736 concentric shells trajectories for ultra fast fMRI. *Magn Reson Med* 68:484-494.
- 737 Zelano C, Jiang H, Zhou G, Arora N, Schuele S, Rosenow J, Gottfried JA (2016) Nasal respiration entrains human
 738 limbic oscillations and modulates cognitive function. *J Neurosci* 36:12448-12467.
- 739
 740
 741
 742
 743
 744
 745
 746
 747
 748
 749
 750
 751

752 **Figure legends**

- 753 **Figure 1. Data processing and exclusion of subjects.** 25 subjects were recruited and scanned in the study. The
 754 final analyses included 15 and 12 subjects, whose results are presented in the manuscript.
- 755
 756 **Figure 2. Study protocol and exemplary signals of awake state and NREM sleep.** a. Study design through one
 757 week of sleep monitoring. Awake scanning session was performed on day two after a good night's sleep. Sleep
 758 scanning session was performed on day five, the morning after a night of documented sleep deprivation. At the
 759 beginning of both sessions, subjects underwent a CANTAB test to evaluate reaction times. In the Awake scan
 760 session, two scans were recorded: I) eyes open (A_{1-2}) and II) eyes closed (EC). In the Sleep scan session two scans
 761 were taken: I) ten-minute Sleep scan (S_{1-2}) and II) ten-minute Sleep scan (S_{3-4}). Numbers (A_{1-2} , S_{1-4}) corresponding
 762 to five-minute segments that were treated separately for further analysis. Percent represents the average fraction of

763 sleep during the scan segment. b-e. Examples of electrophysiological and dynamic brain pulsations measured in the
 764 same subject while awake (100% awake) and sleeping (80% NREM stage 2, 20% NREM stage 1) for five-minute
 765 recordings. Thus, the power spectra were calculated for an entire five-minute segment. The data were measured
 766 simultaneously by b-c. 256 lead high density DC-EEG (Oz) and d-e. fast 10 Hz MREG covering the whole brain in
 767 the ≤ 5 Hz band. Both time and frequency domain data indicated increased power and amplitude of brain pulsations
 768 upon transition from the EEG-verified fully awake state to NREM sleep. The dominant increase in very low
 769 frequency pulsation power reshapes the power spectrum distribution and alters the spectral entropy of the EEG and
 770 MREG signals.

771 **Figure 3. Spectral entropy of MREG decreases in line with increased amount of NREM sleep.** a. EEG sleep
 772 state data show that the amount and depth of sleep both increase as a function of scan segment. b. Visual cortex
 773 SE_{ROI} of MREG data predicts sleep and wakefulness across subjects ($n = 12$). Results indicate linear declines both
 774 in EEG sleep state and MREG SE_{ROI} values as a function of scan segment in the experiment. c. Spectral entropy
 775 decreased significantly in posterior brain areas ($p < 0.05$, $df 11$). d. The SE_{ROI} showed a drop in all subjects in the
 776 transition from wakefulness to EEG-verified sleep states in S_2 . f. The ROC curve of SE_{ROI} data indicate high
 777 accuracy $AUC = 0.88$ ($p < 0.0001$) in the ability to distinguish sleep ($n = 30$ Sleep segments) from awake data ($n =$
 778 30 Awake segments). The model has a sensitivity of 93% and a specificity of 77%.

779 **Figure 4. NREM sleep sharply changes the power spectrum of physiological brain pulsations.** Global MREG
 780 power spectra and power sum difference (paired t-test) between Awake and Sleep in: a. very low frequency (0.008-
 781 0.1 Hz), b. respiratory frequency (0.11-0.44 Hz), and c. cardiac frequency (0.52-1.6 Hz) bands. The global very low
 782 and respiratory frequency power both increased markedly in sleep, and both differed significantly in posterior brain
 783 regions. The global cardiac frequency power increased to a lesser extent, but showed decrease in heart rate ($p <$
 784 0.01 **, $p < 0.05$ *, $df 11$).

785 **Figure 5. Spatial distribution of spectral brain pulsation entropy and power are linked to frequency band.**
 786 Paired t-test shows differences in Sleep versus Awake state ($p < 0.05$, $df 11$). Thresholded mean maps across
 787 subjects and difference maps are presented. A. spectral entropy and b. spectral power in each pulsations frequency
 788 band in five-minute segments in NREM sleep. The three rows show results in very low, respiratory and cardiac
 789 frequencies, respectively. The spatial overlap of the power and entropy increases diminishes as a function of

frequency, with largest overlap in the very low frequency range, partial overlap in the respiratory band, and no overlap in cardiac frequencies (no change in spectral entropy).

Figure 6. EEG slow oscillation (0.2-2 Hz) power increased in spatially overlapping regions in very low, respiratory and cardiac frequency pulsations in MREG. a. EEG slow oscillation (0.2-2 Hz) power sum mean maps in Awake and Sleep, and difference (Sleep > Awake, $p < 0.05$). EEG power maps were converted to the radiological perspective for comparison with MREG results. b. Power sum of MREG pulsations in very low frequency (0.008-0.1 Hz), c. respiratory frequency (0.11-0.44 Hz) and d. cardiac frequency (0.52-1.6 Hz), Sleep > Awake, $p < 0.05$. e. Spectral entropy of MREG in very low frequency (0.008-0.1 Hz), Sleep < Awake, $p < 0.05$. Mean maps between subjects during Awake and Sleep are placed on the left and difference maps on the right column.

Tables

Table 1. The amount of NREM sleep was higher during the Sleep scan session than in the Awake scan session. Mean $\pm$ standard deviation (%) of the amounts of wakefulness, NREM and total sleep times were calculated from the number of sleep-scored EEG epochs in each condition. The highest amount of sleep was achieved in S₂ (mean across subjects 87%). Unknown epochs are likely due to artefacts. A₁ (Awake 1 time segment, five min); A₂ (Awake 2 time segment, five min); EC (Eyes closed, five min); S₁ (Sleep 1 time segment, five min); S₂ (Sleep 2 time segment, five min); S₃ (Sleep 3 time segment, five min); S₄ (Sleep 4 time segment, five min). N values represent the number of totally 15 subjects who had available EEG for sleep scoring.

	Awake scan session			Sleep scan session			
Mean $\pm$ STD	A ₁	A ₂	EC	S ₁	S ₂	S ₃	S ₄
(%)	n=12	n=12	n=13	n=12	n=12	n=10	n=10
Wake	99 $\pm$ 3	99 $\pm$ 3	68 $\pm$ 39	22 $\pm$ 19	11 $\pm$ 23	23 $\pm$ 32	22 $\pm$ 35
N1	1 $\pm$ 3	1 $\pm$ 3	28 $\pm$ 39	47 $\pm$ 25	40 $\pm$ 33	40 $\pm$ 22	35 $\pm$ 30
N2	0	0	4 $\pm$ 14	25 $\pm$ 19	46 $\pm$ 38	37 $\pm$ 29	37 $\pm$ 36
N3	0	0	0	0	1 $\pm$ 3	0	0

No REM sleep was observed.

NREM	sleep							
(%)	1 ± 3	1 ± 3	32 ± 40	72 ± 17	87 ± 21	77 ± 30	72 ± 35	

Table 2. Respiratory and cardiac frequency ranges were determined from physiological EtCO₂ and SpO₂ signals. Individually determined minimum (min) and maximum (max) values for lower and upper edge of the power peak were used for the further analysis. Respiratory rate and heart rate decreased in NREM sleep versus awake based on the power peaking value.

		Respiratory EtCO ₂ (Hz)		
		Min	Peak	Max
Eyes open	n=14	0.17 ± 0.06	0.27 ± 0.06	0.35 ± 0.05
Eyes closed	n=13	0.13 ± 0.06	0.24 ± 0.06	0.32 ± 0.05
Sleep scan 1	n=14	0.17 ± 0.05	$0.24 \pm 0.04^*$	0.31 ± 0.05
Sleep scan 2	n=12	0.16 ± 0.05	$0.24 \pm 0.05^*$	0.32 ± 0.06
		Cardiac SpO ₂ (Hz)		
		Min	Peak	Max
Eyes open	n=14	0.96 ± 0.10	1.07 ± 0.12	1.19 ± 0.16
Eyes closed	n=13	0.90 ± 0.12	$1.03 \pm 0.13^{***}$	1.19 ± 0.18
Sleep scan 1	n=14	0.81 ± 0.13	$0.94 \pm 0.16^{**}$	1.09 ± 0.17
Sleep scan 2	n=11	0.82 ± 0.12	0.94 ± 0.16	1.11 ± 0.21

a. Study protocol

Sleep tracking							
Day1	Day2			Day3	Day4	Day5	
	4-6 PM Awake scan session					6-8 AM Sleep scan session	
	Cantab test				Night4 Sleep deprivation	Cantab test	
		Scan 1	Scan 2			Scan 1	Scan 2
		A ₁ 1(%)	A ₂ 1(%)			S ₁ (72%)	S ₂ (87%)
			EC (32%)			S ₃ (77%)	S ₄ (72%)

b. Awake EEG

c. Sleep EEG

d. Awake MREG

e. Sleep MREG

a. EEG sleep state

b. Spectral entropy ROI

c. Spectral entropy: Sleep < Awake

d. Individual decrease

e. Spectral entropy accuracy for sleep detection

a. Spectral entropy

b. Spectral power