Brain Topography

Resisting Sleep Pressure: Impact on Resting State Functional Network Connectivity

--Manuscript Draft--

Manuscript Number:	BTOP-D-16-00165R1
Full Title:	Resisting Sleep Pressure: Impact on Resting State Functional Network Connectivity
Article Type:	Original Article
Section/Category:	Cognitive neuroscience
Keywords:	BOLD; pseudo BOLD; arterial spin labeling; cerebral blood flow; time awake; independent component analysis; vigilance; imaging modality
Corresponding Author:	Peter Achermann, Ph.D. University of Zurich Zurich, SWITZERLAND
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Zurich
Corresponding Author's Secondary Institution:	
First Author:	Laura Tüshaus, PhD
First Author Secondary Information:	
Order of Authors:	Laura Tüshaus, PhD
	Joshua Henk Balsters, PhD
	Anthony Schläpfer, MSc
	Daniel Brandeis, PhD
	Ruth O'Gorman-Tuura, PhD
	Peter Achermann, Ph.D.
Order of Authors Secondary Information:	
Funding Information:	Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung (CRSII3_136249) — Prof. Peter Achermann
Abstract:	In today's 24/7 society, sleep restriction is a common phenomenon which leads to increased levels of sleep pressure in daily life. However, the magnitude and extent of impairment of brain functioning due to increased sleep pressure is still not completely understood. Resting state network (RSN) analyses have become increasingly popular because they allow us to investigate brain activity patterns in the absence of a specific task and to identify changes under dif-ferent levels of vigilance (e.g. due to increased sleep pressure). RSNs are commonly derived from BOLD fMRI signals but studies progressively also employ cerebral blood flow (CBF) signals. To investigate the impact of sleep pressure on RSNs, we examined RSNs of participants under high (19 h awake) and normal (10 h awake) sleep pressure with three imaging modalities (arterial spin labeling, BOLD, pseudo BOLD) while providing confirmation of vigilance states in most conditions. We demonstrated that CBF and pseudo BOLD signals (measured with arterial spin labeling) are suit-ed to derive independent component analysis based RSNs. The spatial map differences of these RSNs were rather small, suggesting a strong biological substrate underlying these networks. Interestingly, increased sleep pressure, namely longer time awake, specifically changed the functional network connectivity (FNC) between RSNs. In summary, all FNCs of the DMN with any other network or component showed increasing effects as a

	function of increased 'time awake'. All other FNCs became more anti-correlated with increased 'time awake'. The sensorimotor networks were the only ones who showed a within network change of FNC, namely decreased connectivity as function of 'time awake'. These specific changes of FNC could reflect both compensatory mechanisms aiming to fight sleep as well as a first reduction of consciousness while becoming drowsy. We think that the specific changes observed in functional network connectivity could imply an impairment of information transfer between the affected RSNs.

We thank the reviewers for their valuable input. We are glad to see that they perceived our results as interesting and relevant. We provide an extensive point-by-point response below, summarize the changes made, and highlight the changes in the updated manuscript. The text in quotation marks and italics refers to the manuscript.

Reviewer #1:

Main comment:

- **The main limitation of the study lies in the differences between acquisition protocols for the high and low sleep pressure group. For instance, arterial spin labeling sequences were acquired with alternating 'eyes closed' and 'eyes open' periods in the low sleep pressure group, but with eyes open in the high pressure group; the low pressure group did not wear an EEG cap during this sequence but the high pressure group did, and there were differences in tasks that were carried out before the sequences. Although the authors tried to control for these factors and discuss these limitations, it remains unclear how much these differences affected the results. Also, some subjects were excluded because they fell asleep, but there was no possibility to verify whether the low pressure group fell asleep during the arterial spin labeling sequence because EEG was not recorded.**

  Answer:
  We are aware of these limitations in the study design and tried to control for them in the best way possible. Because we took measures as e.g. adding nuisance regressors for the eyes open/closed patterns as well as motion parameters, and because we only included subjects in the NSP group that did not fall asleep in the first recording, displayed normal levels of daytime sleepiness values (Epworth sleepiness scale) as well as normal levels of acute sleepiness (Karolinska sleepiness scale), we are confident that the remaining confounding effects should be small and should not influence our results to a large extent.
  "Further, our results were limited by the absence of EEG recordings in the NSP group during the CBF session. We cannot rule out minor intrusions of sleep in this group during the CBF recordings. However, as these subjects were supposedly well-rested (which was reflected both by their Epworth sleepiness scores – a measure of daytime sleepiness – and the assessment of their acute sleepiness with the Karolinska sleepiness scale; see Tab. 1), were recorded through-out the daytime, did not fall asleep during the preceding BOLD session and after the recording did not report any sleep intrusions, we are confident that this potential confound is small.
  Another limitation stems from the differences regarding the eyes open or closed conditions in the two recording protocols, between the BOLD (2 min EO, 2 min EC, 2 min EO, 2 min EC; Fig. 1) and CBF sessions (HSP: 10 min EO, NSP: 5 min EO, 5 min EC; Fig. 1). Although studies have reported differences in functional connectivity with respect to voluntary eye movements in sleep-deprived subjects (Ong et al. 2015) and vigilance fluctuations (Wang et al. 2016), the reported functional connectivity changes were distinctly different from our observations. Also, since the pattern of cued EO/EC was added as a covariate of no interest in our FNC analysis, this confound should be minimal." (Discussion, p. 15-16)

  The highlighted sections have been added to the manuscript.

Minor comments:

- **The presentation of results in the abstract is not specific enough. For example, the direction of change of FNC in RSN is not mentioned, which makes the conclusion difficult to follow. The methodological aim should be mentioned because this aspect appears as a conclusion.**

  Answer:
  As we observed changes of FNC in different directions (ranging from increased positive correlations over increased negative correlations, to changes from negative to positive correlations and vice versa and decreased negative correlations), we found including detailed descriptions of the change direction too overwhelming for the abstract.
  However, to include some aspect of the observed changes, we changed the following section of the abstract:
  "Interestingly, increased sleep pressure, namely longer time awake, specifically changed the functional network connectivity (FNC) between RSNs. In summary, all FNCs of the DMN with any other network or component showed increasing effects as a function of increased 'time awake'. All other FNCs became more anti-correlated with increased 'time awake'. The sensorimotor networks were the only ones who showed a within network change of FNC, namely decreased connectivity as function of 'time awake'.
  These specific changes of FNC could reflect both compensatory mechanisms aiming to fight sleep as well as a first reduction of consciousness while becoming drowsy. We think that the specific changes observed in functional network connectivity could imply an impairment of information transfer between the affected RSNs." (Abstract, p. 2)

  The highlighted sections have been added to the manuscript.

- ***It is not totally clear to me why the decreasing FNC between RSN could reflect compensatory mechanisms aiming to fight sleep, this main hypothesis should be explained better in the discussion, and backed up with more references/background.***

  Answer:
  Our hypothesis is that the change of FNC from an anti-correlation between attentional networks and the DMN component could represent compensatory mechanisms to maintain wakefulness. This is on the one hand based on several studies that reported decreased anti-correlations between these networks after sleep deprivation (that only included awake subjects), and one study in particular that observed larger reductions of these anti-correlations in subjects more resilient to sleep deprivation. As we specifically included subjects that could maintain wakefulness longer, our sample might be biased towards more resilient subjects.
  "In the specific case of the increase of FNC between the left executive control ATT component and a DMN component, this change from an anti-correlation to a positive correlation with increased time awake might have resulted in a loss of attention (maybe paralleled by a decrease of consciousness) due to the strenuous burden of maintaining wakefulness while struggling not to fall asleep. This is in line with results of several studies that reported decreased anti-correlations between ATT networks and the DMN after sleep deprivation (e.g. De Havas et al. 2012; Yeo et al. 2015; Kaufmann et al. 2016). Yeo and colleagues (2015) described that subjects more resilient to sleep deprivation exhibited relatively larger reductions of the anti-correlations between default mode and attentional

networks. This could hint towards compensatory mechanisms of the brain counteracting the increased need for sleep." (Discussion, p. 12)

This would be in line with the lack of change in FNC involving the thalamus:
"Surprisingly, we did not see any change of FNC involving the subcortical component (SUB, which includes the thalamus). The thalamus is hypothesized to have an influence on arousal levels (which are distinct from attention; Portas et al. 1998; Fan et al. 2005) and would be expected to show changes in connectivity in relation to different levels of sleep pressure. However, Chee and Tan (2010) reported that thalamic levels of activation in subjects not vulnerable to sleep deprivation were unchanged after sleep deprivation. Again, this could reflect compensatory mechanisms attempting to counteract increased sleep pressure in these subjects. In the present study, since we included those subjects who best were able to maintain wakefulness, our observed lack of FNC changes in the SUB component with time awake could be due to a potential bias towards subjects less vulnerable to sleep restriction or loss (see also subsection 'Spatial maps of RSNs'). This might also be the reason why both our study and the study of Kaufmann and colleagues (2016) did not observe any within DMN changes of connectivity. Therefore, the observed changes in FNC between components of RSNs due to 'time awake' that we observed could be a property of sleep restricted subjects who are able to maintain wakefulness under levels of elevated sleep pressure in a challenging environment to stay awake, i.e. those who might be less vulnerable to sleep restriction or loss because their brain is able to compensate." (Discussion, p. 13)

The highlighted sections have been added to the manuscript.

- **Also, the potential utility of using ASL sequences to derive BOLD should be clarified in the discussion, as this appears to be one of the main conclusions of the paper**

Answer:
As ASL recordings to derive functional cerebral blood flow (CBF) are increasing (because these measures provide an absolute value in contrast to the relative nature of the BOLD signal), it was important to us to evaluate the suitability of CBF signals for ICA-derived resting state networks. To address this issue, we did not only want to compare CBF and BOLD signals (that have different sensitivities to specific tissues) but to include a measure that should demonstrate similar tissue sensitivity (in order to rule out differences stemming only from this background) – hence the use of the pseudo BOLD (pBOLD) signals.
"As the BOLD and ASL signals measure quite different phenomena and were not recorded simultaneously (and with different acquisition times (TRs)), we included a "pseudo BOLD" measure derived from the ASL data (presenting a similar tissue sensitivity as BOLD; Wong et al. 1997) in order to address whether differences between ASL and BOLD would be caused by variability in tissue sensitivity.
…
Thus, in this analysis, we investigated RSNs found with ICA of combined BOLD/CBF/pseudo BOLD signals in subjects under high (awake for approximately 19 hours) and normal sleep pressure (awake on average for approximately 10 hours) to verify that CBF signals present a viable option for RSN analyses. Based on previous reports (Sämann et al. 2010; De Havas et al. 2012; Yeo et al. 2015), we expected a decrease in functional net-work connectivity within and between network groups due to increased sleep pressure." (Introduction, p. 4)

In the conclusion we state our aim that these CBF and CBF-derived signals are suited for the kind of resting state network analyses employed in this study.
"Additionally, we demonstrated that CBF and pBOLD signals are suited to derive ICA-based RSNs and that differences between CBF and BOLD measures were rather small, both with respect to the spatial intensity of the RSNs as well as their between network connectivity (i.e. FNC). Therefore, we could verify that CBF signals are indeed a viable option for these types of analyses. As we find the same networks with different modalities, a strong biological substrate must be underlying these resting state networks." (Conclusions, p. 17)

Thus, we think our objectives have been made clear, i.e. by our use of a pBOLD measure, we were able to justify the use of CBF signals for ICA-derived resting state networks.
The highlighted sections have been added to the manuscript.

- **There is a problem in the way the references are displayed in the text (for many of them there is an error message in brackets but not the reference)**

Answer:
We apologize for this inconvenience. Although we took care to remove the EndNote field codes before uploading the manuscript, somehow the Word document was corrupted, resulting in this error. The references have been checked and inserted correctly now.

Reviewer #2:

- **This manuscript is concerned with the impact of the sleep pressure on the resting state brain activity. There is no doubt on the relevance of this topic, with a definitive impact in neuropsychology (impairment of the brain functioning with reduced vigilance, ...). The review of the manuscript was oscillating between excitation and frustration: some imprecisions or apparent methodological flaws weak the present work, and the outcomes are mitigated. The present review will tend to help to clarify some points although the reviewer thinks the main difficulty here is the small size of the cohorts in this experiment.**

Answer:
The size of our cohorts is in a typical range for resting state studies, and in a medium to large range with respect to sleep studies. For example, in the paper by Blautzik et al. Neuroimage 2013 "Classifying fMRI-derived resting-state connectivity patterns according to their daily rhythmicity" where the authors could demonstrate that the degree of daily modulation of resting state networks ranges from highly rhythmic to stable networks, only 15 participants were recorded. Therefore, we are confident that our study cohorts represent a reasonable sample in terms of size and allow us to reliably detect time-awake dependent differences in functional network connectivity.

- **The use of the pBOLD is interesting but the rationale of assessing functional connectivity through CBF is not clear. The fact that the three modalities lead to similar RSNs may be not so surprising given the strong change in brain state induced by the sleep pressure. The authors are fair: it is clear that this study was not planned in the**

original design of the experiment and they clearly explain the limitations of the results that they are discussing.

As ASL recordings to derive functional cerebral blood flow (CBF) are increasing (because these measures provide an absolute value in contrast to the relative nature of the BOLD signal), it was important to us to evaluate the suitability of CBF signals for ICA-derived resting state networks. To address this issue, we did not only want to compare CBF and BOLD signals (that have different sensitivities to specific tissues) but to include a measure that should demonstrate similar tissue sensitivity (in order to rule out differences stemming only from this background) – hence the use of the pseudo BOLD (pBOLD) signals. Further, ASL measures provide absolute values of CBF, thereby allowing a comparison without the need for a baseline recording across scanners and subjects in a way not possible with the BOLD technique.

"Another measurement technique used less in functional imaging up to now is arterial spin labeling (ASL) that measures cerebral blood flow (CBF; Williams et al. 1992). Currently, there are only a few studies that have investigated RSNs with ASL (for a recent review see Chen et al. 2015), possibly due to the lower temporal resolution of ASL compared to BOLD or the smaller signal to noise ratio (Wu et al. 2010). One of the great advantages of ASL, however, is its sensitivity to changes in CBF in small arteries, capillaries and brain parenchyma (Wong et al. 1997). In addition, the capability of this technique to measure absolute changes of CBF (e.g. Aslan et al. 2010) – thereby allowing valid comparisons across subjects and scanners without potential confounds due to differences in baseline measures – might be the cause for the growing number of studies employing ASL. This advantage is particularly important for resting state studies, where no task conditions are available to remove baseline effects by computing differential responses. Also, ASL measurements do not face the so-called "draining vein" problem that can occur in BOLD recordings: due to a draining vein and the BOLD sensitivity mainly to veins, BOLD activation may occur in a brain region downstream from the actual site of neuronal activity (Buxton 2002).

…

As the BOLD and ASL signals measure quite different phenomena and were not recorded simultaneously (and with different acquisition times (TRs)), we included a "pseudo BOLD" measure derived from the ASL data (presenting a similar tissue sensitivity as BOLD; Wong et al. 1997) in order to address whether differences between ASL and BOLD would be caused by variability in tissue sensitivity.

…

Thus, in this analysis, we investigated RSNs found with ICA of combined BOLD/CBF/pseudo BOLD signals in subjects under high (awake for approximately 19 hours) and normal sleep pressure (awake on average for approximately 10 hours) to verify that CBF signals present a viable option for RSN analyses. Based on previous reports (Sämann et al. 2010; De Havas et al. 2012; Yeo et al. 2015), we expected a decrease in functional network connectivity within and between network groups due to increased sleep pressure." (Introduction, p. 4)

Our explanation for the similarity of the resting state networks derived of three different modalities would be that resting state networks exist due to a strong biological substrate which overcomes the specific modality differences.

"Additionally, we demonstrated that CBF and pBOLD signals are suited to derive ICA-based RSNs and that differences between CBF and BOLD measures were rather small, both with respect to the spatial intensity of the RSNs as well as their between network connectivity (i.e.

FNC). As we find the same networks with different modalities, a strong biological substrate must be underlying these resting state networks." (Conclusion, p. 17)

We do not think that this effect is due to some kind of "masking" based on the "strong change in brain state induced by the sleep pressure" as we only found minor difference in the spatial maps of the RSNs due to the factor 'time awake'.
"Only minor differences in the spatial map intensities were due to 'time awake', indicating that the intrinsic networks structures were largely independent of the level of sleep pressure." (Discussion, p. 14)

The highlighted section has been added to the manuscript.

- **Beside the size of the groups, this work suffers from the absence of female in the HSP. Given the sex ratio in the NSP group, this is a problem that the authors do not ignore. However, the reviewer does not understand how interaction with sex can be addressed in this extreme unbalanced situation. Moreover, can we believe that differences of results with Kaufmann et al. can be partially due to this male/female ratio? (only male in Kaufmann et al.)**

  Answer:
  We addressed the potential confounding factor of the sex bias in our study populations with an additional analysis where we included the factor 'sex'. As stated in the Results, Discussion and supplementary material, although this factor slightly influenced the FNC with respect to 'time awake', the general pattern stayed similar to our original analysis. Further, even in large cohort studies with balanced sex ratios, influences on FNC were sparse or absent.
  "As we had a sex bias (some females only in the NSP group), we performed an additional analysis with the covariate 'sex' to address this unbalanced distribution as good as possible. This covariate influenced the FNC due to 'time awake'. However, the overall ratio of females to males was roughly 1:2, therefore, males dominated. Also, all females were awake for a shorter time, and thus had a lower sleep pressure. This bias might lead to shared variance of 'time awake' and 'sex' (though no interaction effect of 'sex' and 'time awake' was observed regarding FNC), and thereby could influence the FNC results. As Online Resource 1 Figure S4 shows, the pattern of FNC due to time awake stayed similar when taking 'sex' into account. As in general sex differences in FNC were relatively sparse or even absent in large-cohort studies (Weissman-Fogel et al. 2010; Allen et al. 2011a; Filippi et al. 2013; Hjelmervik et al. 2014) we think a pure sex influence is rather unlikely and therefore negligible." (Limitations/Advantages, p. 16)

  The highlighted sections have been added to the manuscript.

- **The functional network connectivity has been assessed with the GIFT toolbox. The reviewer suggests the redaction of an appendix that describes the MANCOVAN approach applied to the present case (sensitivity to inter-individual differences?). In general, the sections 'Network identification using ICA' and 'Functional Network Connectivity' were not clear enough to be reproducible and convincing. Another issue not addressed here is about motion correction that may be more subtle for the HSP group. How this correction affects the ICA processing and results?**

Answer:
We added a reference to the GIFT/MANCOVAN manual to the methods section as a detailed description of these methods is beyond the scope of this paper.
"The analysis of functional network connectivity (FNC) was assessed with the MANCOVAN toolbox implemented in the GIFT toolbox (http://mialab.mrn.org/software/gift/docs/v4.0b_gica_manual.pdf; Allen et al. 2011a)." (Materials and methods, p. 8)

Motion correction has been taken into account and addressed. As the GIFT authors advise against motion correction prior to the ICA, we did not apply this step beforehand, but excluded components that resembled artifacts.
"Resting state network components were identified with the GIFT toolbox (http://mialab.mrn.org/software/gift/; http://mialab.mrn.org/software/gift/docs/v4.0b_gica_manual.pdf; Allen et al. 2011a) based on preprocessed data of all subjects (i.e. NSP and HSP subjects pooled) and all imaging modalities (group independent component analysis, group ICA). Twenty-five independent components (ICs; i.e., RSNs) were assumed, based on dimension estimation with minimum description length of the data (Li et al. 2007), visual inspection of the resulting ICs and number of ICs typically used in the literature (e.g. Damoiseaux et al. 2006; Sämann et al. 2011).
Motion correction was not applied before the ICA, as this could influence the FNC analysis. Therefore, selection of components and identification of artifacts was performed by visual inspection, guided by spatial correlation of the components to the data set published by Allen et al. 2011a. Seventeen ICs were identified (Fig. 2; Tab. 2)." (Materials and methods, p. 7)

For the analysis of FNC, we added a regressor of no interest comprising the movement parameters of the realignment step, to reduce effects due to movements.
"For the analyses of FNC, additionally, regressors of no interest were added to reduce their influence on the analysis. These regressors were the movement parameters (derived from the realignment step), EO/EC "condition" (different for CBF/BOLD sessions) and sleep scorings (derived from the simultaneous EEG recordings in all sessions of the HSP group or the BOLD session of the NSP group; to account for different levels of vigilance). The movement parameters did not differ between the NSP and the HSP group (two-way repeated measures ANOVA with factors 'group' and 'modality' performed on the framewise displacement mean values derived from the movement parameters; see Power et al. 2012) but were significantly different for the factor 'modality' ($p < 0.015$). This probably is due to the different sampling rates of the two modalities, that may influence the precision of the movement parameters." (Materials and methods, p. 8)

Also, the aspect of inter-individual differences has been addressed in this section.
"Group ICA provides the advantage that common RSN components are derived from the pooled data and back-projected to each subject. This method has been shown to be sensitive to inter-individual and group differences in these RSN components (Calhoun et al. 2001; Allen et al. 2011b; Erhardt et al. 2011). Further, it circumvents the potential issue of incomparable components arising when performing separate ICAs for each group." (Materials and methods, p. 8)

An additional analysis on group differences of motion parameters has been performed (see above). The highlighted sections have been added to the manuscript.

- **Are the correlations displayed in fig.3 associated to a particular 'time awake' condition? What is the meaning of the indices along the diagonal of the displayed matrix ?**

  Answer:
  These are the mean FNC correlations after correction. Therefore, they are computed across both groups and not attributed to a 'time awake' condition. The indices along the diagonal are indicating the total number of components used in the analysis. As they are not necessarily needed and seem to confuse the reader, we removed them and indicate the diagonal with a dotted line (see new Fig. 3).
  "Mean functional network connectivity (FNC) matrix. Pairwise partial correlations between ICs after correction for movements, eyes open/closed and sleep scores are illustrated. R-values were Fisher z-transformed and averaged across subjects, then back-transformed for display." (Figure legend 3, p. 18)

- **The reading of the Fig.4 is not easy. What is the color bar variable (minus sign? log_1_0 ??) The related section in the manuscript does not help.**

  Answer:
  Univariate results values are displayed as -sign(t statistic)*log10(p-values). Basically this figure shows the positive and negative p-values of the significant changes on a logarithmic scale.
  "'Time awake' effects on the mean FNC matrix. Univariate test results showing the effect of 'time awake' on FNC. Illustrated are the significant $-sign(t)log_{10}(p)$ values, meaning that the significant positive or negative effects are displayed on a logarithmic scale. Floating-point numbers on the colorbar indicate the FDR-corrected threshold ($\alpha = 0.05$)." (Figure legend 4, p. 18)
  The highlighted sentence has been added to the manuscript and labeling in Fig. 4 has been improved (see new Fig. 4).

- **The reviewer agrees with the author with respect to the differences in the spatial map intensities of the RSNs related to the 'time awake', up to the putative flaws due to the unbalanced sex in the HPS. However, the 'group ICA' deserves more explanation and justification. Unless it represents exaggerated extra works, it would be interesting (and helpful in the interpretation) to consider the available DTI to relate variations (moderator in the DMN connectivity with other network connectivity) in FA with respect to the 'time awake' in this experiment (see Elvsashagen et al., PLoS One 10(5), 2015).**

  Answer:
  We chose the 'group ICA' approach because of the following considerations given by the GIFT toolbox implementers (http://mialab.mrn.org/software/gift/faq.html#Q_single_or_group). When running each subject individually, components are difficult to interpret as they may cluster differently in different subjects. Along the same lines, it is preferable to run one large group ICA rather than individual ICAs on different groups to achieve comparable components. Advantages are

that the statistics can be performed for a given component over subjects and this component can also be visualized for different subjects. We think that we have made these advantages clear in the following section:
"Group ICA provides the advantage that common RSN components are derived from the pooled data and back-projected to each subject. This method has been shown to be sensitive to inter-individual and group differences in these RSN components (Calhoun et al. 2001; Allen et al. 2011b; Erhardt et al. 2011). Further, it circumvents the potential issue of incomparable components arising when performing separate ICAs for each group." (Material and methods, p. 8)

Although the combination of the recorded DTI with resting state network functional connectivity would certainly be of great interest, this would require exaggerated extra work. The DTI data have been recorded for later use but as of right now are not preprocessed and therefore not yet ready to be analyzed. Unfortunately, we currently do not have the capacity to perform these analyses.

- **Can we assess the 'transfer of information' through some 'hubness' descriptors of the functional networks?**

Answer:
The idea of 'transfer of information' is based on the hypothesis of Buzsáki and colleagues regarding EEG studies:
"As hypothesized, transfer of information between different brain areas might happen by coherent oscillations of these brain areas, i.e. networks (Buzsáki et al. 2013). Although this hypothesis was formulated on the basis of the EEG, studies have demonstrated a close relationship of the BOLD signal and specific frequency bands in local field potentials derived from intracranial recordings (e.g. Logothetis et al. 2001; Magri et al. 2012)." (Discussion, p. 12)
'Hubness' is a measure employed in graph-theoretical approaches to characterize small world networks. Although it is certainly possible to derive such a measure of the 'hubness' of a network based on functional connectivity recordings of resting state networks, and although it would be interesting to combine the two ideas of small world network properties and transfer of information, we do not have the means to do that in our analysis.
Merely, we find the concept of information transfer happening via coherent oscillations of different brain areas appealing and reasonable, and could imagine that communication in the brain is taking place this way.

- **Note: many references were not linked in the manuscript.**

Answer:
We apologize for this inconvenience. Although we took care to remove the EndNote field codes before uploading the manuscript, somehow the Word document was corrupted, resulting in this error. The references have been checked and inserted correctly now.

Resisting Sleep Pressure: Impact on Resting State Functional Network Connectivity

Short title: **Sleep Pressure & Functional Network Connectivity**

Laura Tüshaus[1,2,3], Joshua Henk Balsters[4], Anthony Schläpfer[2,5], Daniel Brandeis[2,5,6,7], Ruth O'Gorman-Tuura[7,8], Peter Achermann[1,2,3,7*]

[1] Chronobiology and Sleep Research, Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland
[2] Neuroscience Center Zurich, University of Zurich and ETH Zurich, Zurich, Switzerland
[3] Zurich Center for Interdisciplinary Sleep Research, University of Zurich, Zurich, Switzerland
[4] Neural Control of Movement Lab, Department of Health Sciences and Technology, ETH Zurich, Zurich, Switzerland
[5] Department of Child and Adolescent Psychiatry and Psychotherapy, University of Zurich, Zurich, Switzerland
[6] Department of Child and Adolescent Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim/Heidelberg University, Mannheim, Germany
[7] Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland
[8] Center for MR-Research, University Children's Hospital Zurich, Zurich, Switzerland

*** Correspondence:**
Corresponding Author
acherman@pharma.uzh.ch

Keywords: BOLD, pseudo BOLD, arterial spin labeling, cerebral blood flow, time awake, independent component analysis, vigilance, imaging modality.

Acknowledgements

This study was supported by the Swiss National Science Foundation grant CRSII3_136249. We thank Drs. Andrea Federspiel, Philipp Stämpfli, Roger Lüchinger, Kay Jann, Roland Dürr, Thomas Rusterholz for technical support and Drs. Thomas Koenig, Mara Kottlow, Lars Michels and Leila Tarokh for fruitful discussions. We also would like to thank Ximena Omlin, Angela Aeschbach, Claudia Aschmann, Daniela Buser, Angela Escobar, Lukas Fürer, Jolanda Müller, Johanna Scherer, Nina Schumacher, Michelle Steinemann, Sarah Untersander and Katharina Wellstein for help with the data acquisition.

Abstract

In today's 24/7 society, sleep restriction is a common phenomenon which leads to increased levels of sleep pressure in daily life. However, the magnitude and extent of impairment of brain functioning due to increased sleep pressure is still not completely understood.

Resting state network (RSN) analyses have become increasingly popular because they allow us to investigate brain activity patterns in the absence of a specific task and to identify changes under

different levels of vigilance (e.g. due to increased sleep pressure). RSNs are commonly derived from BOLD fMRI signals but studies progressively also employ cerebral blood flow (CBF) signals.

To investigate the impact of sleep pressure on RSNs, we examined RSNs of participants under high (19 h awake) and normal (10 h awake) sleep pressure with three imaging modalities (arterial spin labeling, BOLD, pseudo BOLD) while providing confirmation of vigilance states in most conditions.

We demonstrated that CBF and pseudo BOLD signals (measured with arterial spin labeling) are suited to derive independent component analysis based RSNs. The spatial map differences of these RSNs were rather small, suggesting a strong biological substrate underlying these networks.

Interestingly, increased sleep pressure, namely longer time awake, specifically changed the functional network connectivity (FNC) between RSNs. In summary, all FNCs of the DMN with any other network or component showed increasing effects as a function of increased 'time awake'. All other FNCs became more anti-correlated with increased 'time awake'. The sensorimotor networks were the only ones who showed a within network change of FNC, namely decreased connectivity as function of 'time awake'.
These specific changes of FNC could reflect both compensatory mechanisms aiming to fight sleep as well as a first reduction of consciousness while becoming drowsy. We think that the specific changes observed in functional network connectivity could imply an impairment of information transfer between the affected RSNs.

Introduction

In today's 24/7 society, sleep restriction or curtailment of sleep caused by "social jetlag" (Wittmann et al. 2006) is a common phenomenon. Insufficient sleep may lead to cognitive deficits (e.g. Berger and Oswald 1962; Dinges et al. 1997; Choo et al. 2005; for reviews see Banks and Dinges 2007; Basner et al. 2013) but sleep deprived people are still able to function at a basic level, implying that compensatory mechanisms may play a role in maintaining cognitive function (Wilkinson 1961; Webb and Levy 1984; Horne and Pettitt 1985; Hockey et al. 1998; Drummond et al. 2004). Reduced activation in multiple brain regions following acute total sleep deprivation are commonly observed (see recent meta-analysis of neuroimaging studies by Ma et al. 2015). For example, several studies have demonstrated that acute total sleep deprivation exerts detrimental effects on parietal activation during tasks requiring attention, independent of the specific type of attention (e.g. Drummond et al. 2001; Drummond et al. 2005; Chee et al. 2008; Mander et al. 2008; Tomasi et al. 2009; Chee et al. 2010; Chee and Tan 2010; Lim et al. 2010; Jackson et al. 2011; Czisch et al. 2012; Kong et al. 2012; Muto et al. 2012), and that some parts of the parietal lobe are crucial for the modulation of attention after sleep deprivation (Chee and Choo 2004; Mu et al. 2005; Chee et al. 2006; Lim et al. 2007). Seed-based functional connectivity studies have shown changes in network size and connectivity dependent on the level of sleep pressure (Sämann et al. 2010; De Havas et al. 2012; Yeo et al. 2015), i.e. as a function of the duration of prior wakefulness, and also dependent on the vulnerability to sleep deprivation. These findings could imply an impaired regulation of brain activity after sleep deprivation in specific networks and might be attributed to a misallocation of cognitive resources (more towards networks assumed to be involved in self-referential activity and not attention; see Sämann et al. 2010). Similarly, functional connectivity alterations have been shown in

relation to sleep depth (Horovitz et al. 2009; Larson-Prior et al. 2009; Sämann et al. 2011), suggesting that sustained activity in specific resting state networks is related to the preservation of internal and external awareness but could also play a more general role in integrity maintenance of functional brain systems.

Functional connectivity studies and specifically the investigation of resting state networks (RSNs) with functional magnetic resonance imaging (fMRI) have increased tremendously (e.g. Beckmann et al. 2005; Fox et al. 2005; De Luca et al. 2006; for a recent review on the topic see Raichle 2011), following the seminal paper by Biswal and colleagues (1995). The rise of resting state fMRI may in part be due to ease and speed of acquisition. However, certainly the more important aspect of it is the appealing idea that cognition and perception might arise from cortical oscillation patterns that organize the brain in functional networks (Buzsáki et al. 2013). The absence of a specific task, and short acquisition time (around 10 minutes), make resting state recordings especially desirable for investigating clinical and pediatric populations, as well as altered states of vigilance when tasks cannot be performed (e.g. coma, anesthesia, and sleep). In general, functional connectivity (whether derived from seed-based or independent component analysis (ICA) RSN approaches) groups together brain areas showing a similar pattern of temporal fluctuations (i.e. activation).

Most studies employing fMRI have measured the standard blood oxygen level dependent (BOLD) response. This technique measures the relative changes of oxygenated and deoxygenated blood and is most sensitive for veins and tissue surrounding veins (Wong et al. 1997). Another measurement technique used less in functional imaging up to now is arterial spin labeling (ASL) that measures cerebral blood flow (CBF; Williams et al. 1992). Currently, there are only a few studies that have investigated RSNs with ASL (for a recent review see Chen et al. 2015), possibly due to the lower temporal resolution of ASL compared to BOLD or the smaller signal to noise ratio (Wu et al. 2010). One of the great advantages of ASL, however, is its sensitivity to changes in CBF in small arteries, capillaries and brain parenchyma (Wong et al. 1997). In addition, the capability of this technique to measure absolute changes of CBF (e.g. Aslan et al. 2010) – thereby allowing valid comparisons across subjects and scanners without potential confounds due to differences in baseline measures – might be the cause for the growing number of studies employing ASL. This advantage is particularly important for resting state studies, where no task conditions are available to remove baseline effects by computing differential responses. Also, ASL measurements do not face the so-called "draining vein" problem that can occur in BOLD recordings: due to a draining vein and the BOLD sensitivity mainly to veins, BOLD activation may occur in a brain region downstream from the actual site of neuronal activity (Buxton 2002).

To our knowledge, no study has investigated independent component analysis (ICA) derived RSNs in subjects under different levels of sleep pressure and specifically changes in functional network connectivity within and between these RSNs. Also, only few studies compared the reliability of RSNs derived from CBF and BOLD data (Jann et al. 2013; Jann et al. 2015).
Therefore, we compared resting state fMRI recorded with both the BOLD and ASL techniques. As the BOLD and ASL signals measure quite different phenomena and were not

recorded simultaneously (and with different acquisition times (TRs)), we included a "pseudo BOLD" measure derived from the ASL data (presenting a similar tissue sensitivity as BOLD; Wong et al. 1997) in order to address whether differences between ASL and BOLD would be caused by variability in tissue sensitivity.

Thus, in this analysis, we investigated RSNs found with ICA of combined BOLD/CBF/pseudo BOLD signals in subjects under high (awake for approximately 19 hours) and normal sleep pressure (awake on average for approximately 10 hours) to verify that CBF signals present a viable option for RSN analyses. Based on previous reports (Sämann et al. 2010; De Havas et al. 2012; Yeo et al. 2015), we expected a decrease in functional network connectivity within and between network groups due to increased sleep pressure.

Material and methods

Participants and protocol

In total, data of 45 subjects were recorded in the same scanner and with an identical scanning protocol. Data of 34 participants were combined in the final analysis. Participants were excluded due to the following reasons: 1) falling asleep at the beginning of a scan session (initial 3 minutes; n=1), 2) being asleep >50 % of the recording session (n=1), 3) technical difficulties with the EEG (insufficient quality of the EEG; n=2), 4) lack of a clear alpha rhythm in wake EEG (n=3), 5) motion in one or both sessions of larger than 1 mm (n=2), 6) discordance with the protocol (n=2). Resting state data were recorded after approximately 10 and 19 hours of wakefulness (Fig. 1). The ethical committee of the canton of Zurich approved the study, and it was performed according to the Helsinki declaration. Participants gave their written informed consent and were remunerated for their participation.

Participants were selected for the study after a thorough screening process excluding excessive daytime sleepiness based on standard cutoff (defined as values > 10, assessed by the Epworth Sleepiness Scale (ESS; Johns 1991). Participants reported not having neurological or psychiatric diseases and had a normal body mass index (BMI) according to the WHO (normal range: 18.5 – 24.99 kg/m^2; http://apps.who.int/bmi/index.jsp?introPage=intro_3.html). Demographics and behavioral measures of the participants are provided in Tab. 1.

Protocol

Participants consisted of two groups (normal sleep pressure group, NSP, and high sleep pressure group, HSP; details see below) and underwent the following study protocols (Fig. 1): Assessment of their current sleepiness with the Karolinska Sleepiness Scale (KSS; Åkerstedt and Gillberg 1990; range: 1 – 9, 1: extremely alert, 9: extremely sleepy – fighting sleep), calibration and resting state EEG outside of the scanner (RSout), followed by a resting state recording in the scanner with simultaneous EEG and BOLD-fMRI (RSin). Afterwards, all participants performed a modified (Michels et al. 2010) Sternberg working memory task (SB;Sternberg 1966) and participants in the NSP group some additional tasks (modified

speeded Eriksen Flanker: Kopp et al. 1996; Bunge et al. 2002; Iannaccone et al. 2015) and Stop signal task: Rubia et al. 2003). After a short break, resting state fMRI data were recorded with simultaneous EEG/fMRI-ASL (RSasl; HSP group) or fMRI-ASL (RSasl; NSP group, no EEG). Anatomical images (T1, DTI) were acquired at the end of the respective study protocol without the EEG cap.

In the HSP group the EEG was recorded during both BOLD and ASL measurements, whereas in the NSP group it was only measured during the BOLD session. During the resting state recordings, participants were instructed to focus their gaze on a white fixation cross in the center of their dark field of view, delivered by scanner compatible video goggles when having their eyes open (EO). During the BOLD session, participants alternated between 2 min EO, 2 min eyes closed (EC), 2 min EO, 2 min EC. To lessen the chance of HSP participants falling asleep during the ASL session, they were instructed to keep their eyes open for 10 min, whereas the NSP group had 5 min EO and 5 min EC.

Participants of both groups were similar in terms of age, BMI, daytime sleepiness (ESS) and mid sleep time on free days (corrected for age, gender and sleep debt, assessed by the Munich Chronotype Questionnaire (MCTQ; Roenneberg et al. 2003; Tab. 1).

Normal sleep pressure group (NSP)

Seventeen healthy adults (23.9 ± 2.3 (SD) years; 7m, 10 f) slept normally at home (approximately 8 h) prior to recordings (no sleep restriction the night before, thus not challenged with extended wakefulness) and reported on average habitual sleep durations of 7.6 ± 0.5 h (range: 6.5 -8.4 h). Recordings took place during the day (clock time: 12:23 – 18:36, time awake: 8:06 – 14:23 h). Time awake was calculated from the actual recording times with respect to habitual wake up time derived from the MCQT. Participants of this group showed low to moderate levels of sleepiness (mean: 4.4 ± 2.1, Tab. 1; assessed by the KSS, a validated measure of sleepiness, which is sensitive to acute modifications in sleepiness).

High sleep pressure group (HSP)

Seventeen healthy males (22.4 ± 2.5 years) were recorded in the evening after 19 hours of prior wakefulness (clock time: 20:40 – 21:38, time awake: 17:43 – 20:09 h) and reported on average habitual sleep durations of 7.5 ± 0.6 h (range: 6.4 -8.5 h). Time awake was calculated from the actual recoding times with respect to actual wake up time (3:30 am for all participants of this group). Sleep was restricted to 4 hours time in bed in the sleep laboratory the night prior to scanning and participants adhered to 8 hours time in bed the three nights prior to the recording in the sleep laboratory. Due to the protocol, the participants of this group showed significantly higher levels sleepiness (KSS, mean: 6.7 ± 1.3; Tab. 1).

fMRI and EEG Data Acquisition

The resting state recordings were performed with a 3T Philips Achieva whole-body system (Philips Medical Systems, Best, the Netherlands) with a 32-elements receive-only head coil (Philips SENSE head coil 32-elements).

First, simultaneous EEG/fMRI-BOLD recordings were obtained. BOLD images were acquired with 2D EPI readout with the following parameters: 250 volumes, a 80 x 77 matrix, a slice thickness of 3 mm with a 0.7 mm gap, 3 x 3 x 3 mm^3 isomorphic voxels, 35 slices, a repetition time/time echo (TR/TE) of 1960/30 ms, a flip angle of 80°, and a 240 mm field of view.

Afterwards, the fMRI-ASL recordings (simultaneous with EEG for the HSP group) were conducted. ASL images were obtained with 2D EPI readouts with a pseudo-continuous labeling scheme with the following parameters: 72 volumes, a 80 x 79 matrix, a slice thickness of 7 mm with no gap, 3 x 3 x 7 mm^3 voxels, 20 slices, repetition time/time echo/label time/post label delay (TR/TE/τ/PLD) of 4400/20/1650/1525 ms, a flip angle of 90°, a field of view of 240 mm, a labeling offset of 2 cm from the bottom imaging slice (corresponding roughly to 9 cm offset from the anterior commissure-posterior commissure (AC-PC) line) and background suppression. To quantify CBF (Deibler et al. 2008), an equilibrium magnetization volume (M_0) was acquired right before the fMRI-ASL recordings, with the same parameters as for the fMRI-ASL sequence described above, except that a longer TR of 10000 ms and no labeling was applied. Both BOLD and ASL slices were oriented along the AC-PC line such that the whole cortex was included. Therefore, the cerebellum was not fully covered with the selected slices. From the unquantified ASL images, the pseudo BOLD (pBOLD) images were reconstructed (see below).

Concurrent EEG was acquired with an MR compatible amplifier and electrodes (BrainAmp, BrainCap and BrainAmp ExG MR devices and electrodes; Brain Products GmbH, Gilching, Germany; 60 EEG, 2 EOG, 1 EMG, 3 EKG channels; EEG electrodes placed according to the international 10-20 system, Nuwer et al. 1998). Data were sampled at a 5 kHz and synchronized with the scanner clock (see supplementary Online Resource 1 for more details).

Data processing

fMRI

All fMRI data were first realigned in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). Participants' anatomical T1 images were co-registered to the mean functional image per run and then normalized to MNI space.

BOLD data were normalized to MNI space (by way of the co-registered and normalized T1 image and resliced to 3 x 3 x 3 mm^3 isomorphic voxels), masked and smoothed (6 mm full-width at half maximum (FWHM) Gaussian Kernel) with SPM8.

ASL data were quantified to CBF images (in house MATLAB script courtesy A. Federspiel, University Hospital of Psychiatry Bern; based on the simple subtraction method, i.e. subtracting the mean of the surrounding images from the image in question, described in

Aguirre et al. 2002 as this method has been described to reliably minimize BOLD contamination in resting state recordingsLiu and Wong 2005), normalized to MNI space (by way of the co-registered and normalized T1 image and resliced to 3 x 3 x 3 mm^3 isomorphic voxels), masked and smoothed (6 mm FWHM Gaussian Kernel) with SPM8.

Pseudo BOLD (pBOLD) images were reconstructed from the realigned ASL images, by way of surround addition (adding the mean of the two surrounding images to the image in question, as described inWong et al. 1997), normalized to MNI space (by way of the co-registered and normalized T1 image and resliced to 3 x 3 x 3 mm^3 isomorphic voxels), masked and smoothed (6 mm FWHM Gaussian Kernel) with SPM8. This method has been validated and reveals images comparable to regular BOLD images (Wong et al. 1997).

Variance maps

To assess whether potential modality differences would arise due to differences of variability inherent of the modalities (e.g. higher tissue specificity in one modality but not the other, higher noise in different areas of the brain dependent on modality), we chose to evaluate variance maps of all modalities.

Variance maps were derived from the final preprocessed images of all modalities (after removal of visually identified ICA-derived artefact components and back-projection to subjects' native space with the GIFT toolbox; description of ICA analysis see below). For each subject, the temporal variance of each voxel was calculated and log-transformed. These images were then normalized by subtracting the overall logarithmic mean value of the image, and averaged across subjects.

EEG

The EEG was preprocessed according to standard approaches (i.e., removal of the gradient artifacts by template subtraction, removal of the cardioballistogram artifacts by template subtraction, subsequent ICA across pooled EEG data measured outside and inside the scanner to remove residual artifacts; Allen et al. 1998; Allen et al. 2000; Mantini et al. 2007). EEG was visually inspected for signs of sleep by a trained expert (derivations C3A2, F3A2, O1A2; A2 approximated by closest electrode in the EEG cap, i.e. TP10) in 20-s epochs according to standard criteria (Rechtschaffen and Kales 1968; Iber et al. 2007) and subjects were considered sleeping when stage 2 non-rapid eye movement (non-REM) sleep occurred.

Network identification using ICA

Resting state network components were identified with the GIFT toolbox (http://mialab.mrn.org/software/gift/; http://mialab.mrn.org/software/gift/docs/v4.0b_gica_manual.pdf; Allen et al. 2011a) based on preprocessed data of all subjects (i.e. NSP and HSP subjects pooled) and all imaging modalities (group independent component analysis, group ICA). Twenty-five independent components (ICs; i.e., RSNs) were assumed, based on dimension estimation with minimum description length of the data (Li et al. 2007), visual inspection of the resulting ICs and

number of ICs typically used in the literature (e.g. Damoiseaux et al. 2006; Sämann et al. 2011).

Motion correction was not applied before the ICA, as this could influence the FNC analysis. Therefore, selection of components and identification of artifacts was performed by visual inspection, guided by spatial correlation of the components to the data set published by Allen et al. 2011a. Seventeen ICs were identified (Fig. 2; Tab. 2). Components were numbered according to the output of the GIFT toolbox group ICA. Group ICA provides the advantage that common RSN components are derived from the pooled data and back-projected to each subject. This method has been shown to be sensitive to inter-individual and group differences in these RSN components (Calhoun et al. 2001; Allen et al. 2011b; Erhardt et al. 2011). Further, it circumvents the potential issue of incomparable components arising when performing separate ICAs for each group.

Functional Network Connectivity

The analysis of functional network connectivity (FNC) was assessed with the MANCOVAN toolbox implemented in the GIFT toolbox (http://mialab.mrn.org/software/gift/docs/v4.0b_gica_manual.pdf; Allen et al. 2011a). This analysis takes into account the temporal fluctuations of each component and computes its (partial) correlation with the temporal fluctuations of every other component (including potential regressors of no interest).

Statistics

In order to test the impact of 'time awake' (i.e. continuous variable, not group difference) and 'modality' (i.e., BOLD, CBF, pBOLD), statistical differences were assessed with the MANCOVAN toolbox implemented in the GIFT toolbox (Allen et al. 2011a). We tested 'time awake' rather than a difference between the HSP and NSP group because the NSP group had a larger variance in their time awake (std: 2.3 h, both for the BOLD and ASL session) than the HSP group (std: 0.4/0.5 h for BOLD/ASL session, respectively).
We were interested in two aspects regarding the RSNs: 1) their spatial map intensities and 2) the FNC between RSNs.
Seventeen ICs entered the statistical analysis investigating spatial map intensities with covariates of 'time awake' and 'modality'. For the analyses of FNC, additionally, regressors of no interest were added to reduce their influence on the analysis. These regressors were the movement parameters (derived from the realignment step), EO/EC "condition" (different for CBF/BOLD sessions) and sleep scorings (derived from the simultaneous EEG recordings in all sessions of the HSP group or the BOLD session of the NSP group; to account for different levels of vigilance). The movement parameters did not differ between the NSP and the HSP group (two-way repeated measures ANOVA with factors 'group' and 'modality' performed on the framewise displacement mean values derived from the movement parameters; see Power et al. 2012) but were significantly different for the factor 'modality' ($p < 0.015$). This probably is due to the different sampling rates of the two modalities, that influences the precision of the movement parameters. The partial correlations within and between RSN

groups' time courses were Fisher z-transformed, averaged across subjects and back transformed for visualization (Allen et al. 2011a).
All reported spatial and connectivity effects were significant at the level $p<0.05$ after correction for false discovery rate (FDR). The percentage values reported in Tab. 2 were calculated with respect to the total voxel number of each RSN group (i.e., combining the number of voxels of all ICs forming a network group). In the sensorimotor RSN group e.g., this would mean adding the voxels of the four ICs to achieve the total number and equally combining all voxels with a positive/negative effect of all these ICs, respectively (for details on the RSN groups, see below). They denote the number of voxels in each RSN group showing an effect.

Results

Spatial map intensities of RSNs

Seventeen spatial maps, spatially highly correlated with the data templates provided in the publication of Allen et al. (2011a), were identified, forming seven RSN groups (Fig. 2, Tab. 2): one thalamic and basal ganglia component (IC 7; subcortical network, SUB), an auditory component (IC 4; auditory network, AUD), four components forming the sensorimotor networks (IC 5/12/17/21; SENS), three components comprising visual networks (IC 2/15/18; VIS), four components representing the default mode networks (IC10/20/22/23; DMN), three components forming attentional networks (IC 8/14/24; ATT) and one cerebellar component (IC 16; cerebellar network, CEREB).

Differences in spatial map intensities of RSNs due to 'time awake'

There were only minor effects of 'time awake' on the spatial intensity maps (< 1 % of voxels) and even smaller interaction effects between 'time awake' and 'modality'. To account for the sex differences between the groups, 'sex' was added as a covariate in an additional MANCOVAN analysis. There were only minor effects of 'sex' and an interaction of 'sex' and 'time awake' (< 3 % and approximately 1 % of the voxels, respectively; not shown).

Differences in the spatial map intensities of RSNs due to 'modality'

The topography of the spatial map intensities was similar across modalities but we observed significant differences in intensity (Online Resource 1 Figure S1, Tab. 2).

The largest effect of modality on spatial intensity maps was observed between CBF and BOLD maps (Online Resource 1 Figure S1A and D) with effects largely localized to the SUB (IC 7) and SENS (IC5, IC12, IC17 and IC21) networks (see also Tab. 2).

Differences between the BOLD and pBOLD spatial intensity maps were smaller but still showed some pronounced effects that looked quite similar to the differences between CBF and BOLD spatial intensity maps (Online Resource 1 Figure S1B and E). Apart from the SUB network (26 % of the voxels related to higher pBOLD sensitivity; Tab. 2), spatial

intensity map differences were small comparing pBOLD and BOLD, in the range of 0.2-7 % of the respective RSN groups' voxels (Tab. 2).

Differences between CBF and pBOLD were minimal (Online Resource 1 Figures S1C and F), affecting around 1 % of the respective RSN groups' voxels (Tab. 2).

This suggests that differences between BOLD and the other two modalities may reflect parameters such as e.g. recording voxel size rather than differences in the signals acquired by the different modalities.

To assess whether differences between modalities arose due to high variance caused by one of the modalities in those areas showing modality differences, variance maps of each modality were calculated (Online Resource 1 Figure S2). The largest variance occurred in the CBF signal (Online Resource 1 Figure S2B), most prominently more or less in the subarachnoid space surrounding the brain (following the interhemispheric fissure and close to the pons), as well as to a lesser degree in a prefrontal region of the cortex. The other two modalities (BOLD and pBOLD, Online Resource 1 Figure S2A and C, respectively) showed highly comparable variance distributions, while their highest variance values were present in similar brain areas as for the CBF data, however less pronounced. The variability distribution of the three modalities did not coincide with the areas where the spatial map intensities differed between modalities. This further suggests that the detection intensity differences between the modalities were not caused by high variance of one modality in particular brain areas.

Functional network connectivity between components

Mean FNC (meaning pairwise partial correlations of the time courses of different ICs, i.e. RSNs) resembled the findings from the literature (Allen et al. 2011a): the network components were highly correlated within the RSN groups, especially within the SENS, VIS, DMN and ATT networks (Fig. 3).
Furthermore, the SUB component was associated with the SENS and DMN networks, whereas the AUD network was correlated with SENS, VIS, DMN and ATT networks. Also, the SENS and both the VIS and ATT networks were correlated, and the VIS networks were related to the CEREB component. Both correlations and anti-correlations were observed (Fig. 3).

Interpretation of the direction of functional network connectivity effects

When assessing the FNC between components with regard to the covariates 'time awake' or 'modality', the displayed effects are not so straightforward to interpret and do not necessarily reflect correlations between the components directly. Positive effects can arise from positive correlations increasing with the respective covariate, from anti-correlations becoming less anti-correlated or from a change from an anti-correlation to a correlation. Similarly, negative effects on FNC regarding the respective covariate can be caused by anti-correlations that increase in their negative correlation, weakening of positive correlations or due to the change from a correlation to an anti-correlation. Thus, in Fig. 4 these effects are detailed by arrows.

'Time awake' effects on functional network connectivity

FNC within and between network groups (SUB, AUD, SENS, VIS, DMN, ATT, CEREB) was affected by the amount of time participants were awake before the resting state recording. 'Time awake' was treated as a continuous covariate of interest combining the NSP and HSP group (Fig. 4). However, to facilitate interpretation regarding the direction of change, group average FNC are illustrated (Online Resource 1 Figure S3B and C).

In summary, all FNCs of the DMN with any other network or component showed increasing effects (Fig. 3), either by increasing correlations, weakening anti-correlations or even changing from an anti-correlation to a positive correlation as a function of increased 'time awake'. All other FNCs became more anti-correlated with increased 'time awake', either manifesting in increasing negative correlations, weakening positive correlations, or changes from correlation to anti-correlation with increasing sleep pressure. The SENS networks were the only ones who showed a within network change of FNC, namely a negative effect as function of 'time awake'. More details can be found in the supplementary Online Resource 1 and especially Online Resource 1 Figure S4.

When including 'sex' as a covariate in the additional analysis (Online Resource 1, Online Resource 1 Figure S4), the sex difference between our two groups slightly influenced the 'time awake' FNC. However, there were no significant effects on FNC due to 'sex' alone or any interactions between 'time awake' and 'sex'. The overall pattern of FNC due to 'time awake' stayed similar to the one observed without the 'sex' covariate (Online Resource 1 Figure S4).

Discussion

FNC within and between RSNs

The FNC within and between RSNs regardless of 'time awake' and 'modality' was in good agreement to previous literature (Allen et al. 2011a), displaying high FNC within the network groups, as well as high correlations between ATT and SENS components.

'Time awake' effects on functional network connectivity

FNCs between some of the RSNs and within one RSN were dependent on the duration of the time awake until the data acquisition. All RSN components in which a positive effect with time awake was observed comprised components of the DMN. All changes in FNC not involving any DMN components revealed negative effects of FNC as a function of 'time awake'. As HSP subjects were under considerable sleep pressure and in a demanding environment to stay awake (lying in a dark scanner room with no explicit task), they could have been transitioning between wakeful awareness and drowsiness. This could have resulted in a stronger dominance of the DMN (which is associated with self-referentialism and introspect; Mason et al. 2007; Buckner et al. 2008), as well as an attempt of the ATT component to approximate DMN fluctuations as drowsiness became more prevalent. Along these lines of thought, Poudel and colleagues (2012) found increases of CBF in both the basal

forebrain, as well as in the anterior and posterior cingulate cortex (areas which are part of the DMN) in participants who were able to remain alert after sleep deprivation. In a seed-based BOLD study, although most within-region of interest (ROI) correlations stayed similar throughout light sleep and wakefulness, stronger correlations of ROIs in the supplementary motor areas and right calcarine sulcus were observed during light sleep (Horovitz et al. 2008). Also, Larson-Prior and colleagues (2009) found no change in within-ROI connectivity in the VIS, AUD and SENS as well as the DMN networks during light sleep but increases in the dorsal attention network and decreases in the executive control network (in our analysis comprised of ICs 14 and 24; Fig. 2). These reported changes of seed-based within network connectivity (in the dorsal attention and executive control network) might be paralleled in our findings by changes in network connectivity within the ATT RSNs group. However, as both previous studies (Horovitz et al. 2008; Larson-Prior et al. 2009) looked at light sleep (defined as stage 1 and 2 of non-REM sleep) and subjects were not sleep-deprived prior to scanning, their results are not directly comparable with our findings.

In general, the pattern of FNC changes that we found as a function of 'time awake' is distinctively different from the one observed during sleep, where DMN connectivity along with all other network connectivity decreased with deeper sleep stages (Horovitz et al. 2009; Sämann et al. 2011). Therefore, the state of drowsiness due to increased sleep pressure might be distinctly different from the process of falling asleep and being in light sleep.

As hypothesized, transfer of information between different brain areas might happen by coherent oscillations of these brain areas, i.e. networks (Buzsáki et al. 2013). Although this hypothesis was formulated on the basis of the EEG, studies have demonstrated a close relationship of the BOLD signal and specific frequency bands in local field potentials derived from intracranial recordings (e.g. Logothetis et al. 2001; Magri et al. 2012). Therefore, if the temporal fluctuations in specific networks approached synchronicity with the DMN, their FNC with other networks should decrease as they no longer would exchange meaningful information (as now most of them would fluctuate with the DMN pattern). With an increase of time awake, we observed that 1) networks which previously were positively correlated now showed weak anti-correlations, and 2) that the anti-correlation between the VIS IC and ATT networks decreased, both effects potentially implying reduced information transfer between these networks (along the lines of Buzsáki et al. 2013). The stronger anti-correlation of the left executive control network (which is thought to be involved in cognitive processing; Smith et al. 2009; Laird et al. 2011) and the SENS IC could be due to an within-network change in the SENS FNC with increased time awake. In the specific case of the increase of FNC between the left executive control ATT component and a DMN component, this change from an anti-correlation to a positive correlation with increased time awake might have resulted in a loss of attention (maybe paralleled by a decrease of consciousness) due to the strenuous burden of maintaining wakefulness while struggling not to fall asleep. This is in line with results of several studies that reported decreased anti-correlations between ATT networks and the DMN after sleep deprivation (e.g. De Havas et al. 2012; Yeo et al. 2015; Kaufmann et al. 2016). Yeo and colleagues (2015) described that subjects more resilient to sleep deprivation exhibited relatively larger reductions of the anti-correlations between

default mode and attentional networks. This could hint towards compensatory mechanisms of the brain counteracting the increased need for sleep. Furthermore, it has been suggested that activity in the executive control networks could be a necessary condition for conscious visual perception (Dehaene et al. 2003).Therefore, a decrease of activity in these networks (and with it less pronounced FNC) might be paralleled by a loss of its hypothesized function. Also, most task-based studies investigating sleep restriction or deprivation found changes of brain activation in areas thought to involve executive functioning or attention (for a review see Jackson et al. 2013).

A recent study looking at changes of the brain functional connectome as a function of time of day (morning/evening sessions) as well as total sleep deprivation found very diverse changes of functional network connectivity due to total sleep deprivation: increased positive correlations (between DMN and both VIS and ATT networks as well as AUD and amygdala components), increased negative correlations (between DMN and frontal networks, ATT and VIS components, as well as frontal and CEREB networks), some changes from negative to positive correlations (between DMN and VIS networks, within both ATT and AUD network, between ATT and VIS components, and frontal and SENS networks), and decreased magnitude of negative correlations (between DMN with frontal, ATT and CEREB networks, as well as between ATT and AUD components; Kaufmann et al. 2016). Though their changes in FNC mostly do not resemble the pattern that we observed in our study, this might be due to several factors. Methodologically, their assessment of sleep stages (and thereby also wakefulness) was based on a trained classifier of the fMRI data, and they considered non-REM sleep stage 1 as being asleep. Further, subjects underwent total sleep deprivation prior to the scans. In addition, network components were slightly different and their clustering to networks resulted in other categorization compared to ours (e.g. the left and right executive control networks were assigned to “frontal networks” while we included them in the “attentional networks”). Nevertheless, they also did not report within DMN alterations (Kaufmann et al. 2016). In addition, along our lines, Kaufmann and colleagues (2016) observed, that when trying to classify sessions (morning, rested; evening, rested; morning, rested/sleep deprived) based on FNC data of their sleep deprived group, the morning session after sleep deprivation was classified as being “evening-like”, whereas in their rested control group, the second morning scan was equally classified as being “morning-” or “evening-like”, suggesting an impact of sleep deprivation.

Sämann and colleagues (2010) reported reduced functional connectivity with increased sleep pressure in main nodes of both the DMN and its so-called anti-correlated network in a seed based approach. We did not observe changes along these lines. However, in Sämann et al. (2010), maintenance of wakefulness was only assessed by subjective reports. Furthermore, the changes in functional connectivity were assessed between the nodes that formed a network. In our case, these nodes would probably comprise one IC and changes of functional connectivity within a specific IC would only be visible in differences of the spatial map intensities with time awake. As this was not the case, it might be that the subjects of Sämann et al. (2010) displayed different levels of drowsiness and that the differences found in functional connectivity of the nodes within the DMN and anti-correlated network could have

been affected by intrusion of light sleep (not measured). Also, in the study of Sämann and colleagues (2010), only 14 subjects were included in the analysis.

Surprisingly, we did not see any change of FNC involving the subcortical component (SUB, which includes the thalamus). The thalamus is hypothesized to have an influence on arousal levels (which are distinct from attention; Portas et al. 1998; Fan et al. 2005) and would be expected to show changes in connectivity in relation to different levels of sleep pressure. However, Chee and Tan (2010) reported that thalamic levels of activation in subjects not vulnerable to sleep deprivation were unchanged after sleep deprivation. Again, this could reflect compensatory mechanisms attempting to counteract increased sleep pressure in these subjects. In the present study, since we included those subjects who best were able to maintain wakefulness, our observed lack of FNC changes in the SUB component with time awake could be due to a potential bias towards subjects less vulnerable to sleep restriction or loss (see also subsection 'Spatial maps of RSNs'). This might also be the reason why both our study and the study of Kaufmann and colleagues (2016) did not observe any within DMN changes of connectivity. Therefore, the observed changes in FNC between components of RSNs due to 'time awake' that we observed could be a property of sleep restricted subjects who are able to maintain wakefulness under levels of elevated sleep pressure in a challenging environment to stay awake, i.e. those who might be less vulnerable to sleep restriction or loss because their brain is able to compensate.

Spatial maps of RSNs

We could demonstrate that group ICA with combined CBF, BOLD and pBOLD data detected the same networks (Fig. 2) that have been identified previously with BOLD data (e.g. Damoiseaux et al. 2006; Allen et al. 2011a; Heine et al. 2012). The topography of the spatial intensity maps was very similar across modalities only differing in their detection sensitivity (Online Resource 1 Figure S1, Tab. 2).

Differences in the spatial map intensities of RSNs due to 'time awake'

Only minor differences in the spatial map intensities were due to 'time awake', indicating that the intrinsic networks structures were largely independent of the level of sleep pressure. This might seem counterintuitive at first sight, since several studies showed changes due to sleep restriction or sleep deprivation in specific resting state network areas (Braun et al. 1997; Wu et al. 2006; Sämann et al. 2010; De Havas et al. 2012; Poudel et al. 2012). However, most of these studies used a seed-based approach to define resting state networks and compared the extent of correlated areas with specific seeds. As we used an ICA approach that combines correlated areas into networks, changes within these components or networks cannot be analyzed further. We performed a group ICA (and not two separate ICAs, i.e. one per group), it is thus possible that group differences might have become more apparent with two separate ICAs. However, the group ICA approach we used in this study has been shown to be sensitive to inter-individual variability even in a single group ICA (Allen et al. 2011b). Further, a good test-retest reliability has been attributed to RSNs derived from both seed-based and ICA approaches (Franco et al. 2013). As mentioned in the methods, employing

group ICA approach provides the advantage that the resulting components are present in all subjects and are directly comparable.

Because we explicitly excluded subjects from the analysis that showed strong signs of sleep, we might have chosen subjects that were less vulnerable to sleep restriction, i.e. increased sleep pressure. As mentioned above, Chee and Tan (2010) observed that subjects not vulnerable to sleep deprivation did not show the same patterns of brain activation during a visual attention task as their vulnerable counterparts and that their neural activation characteristics more closely resembled those of non-sleep deprived subjects. Similarly, Poudel and colleagues (2012) observed an overall reduction of CBF in attentional networks that seemed to be driven mostly by subjects who showed strong signs of drowsiness.

Differences in the spatial map intensities of RSNs due to 'modality'

We found some differences in the intensities of the networks due to 'modality' (see supplementary Online Resource 1 as well as Online Resource 1 Figure S1). The majority of differences were found between the not simultaneously obtained modalities with slightly different recording parameters (CBF/BOLD, pBOLD/BOLD) but only minor differences between those recorded simultaneously (CBF/pBOLD). This is along the lines of a recent study showing highly reproducible network-specific CBF measures (Jann et al. 2015).

It therefore seems likely that these differences in spatial map intensities (comparing CBF/BOLD and pBOLD/BOLD) stem mainly from the underlying differences of the recording parameters, though both were resliced to the same spatial resolution and filtered to match sampling rates during preprocessing).

We further ruled out that the modality differences were due to high variability of one of the methods in specific brain areas. Variability distributions were very similar for the three modalities with highest values observed for CBF (Online Resource 1 Figure S2). Brain areas showing differences due to 'modality' did not overlap with the areas of largest variance in the three modalities.

Limitations/Advantages:

A limitation of our study was the lack of a within group design as this was not initially planned. This could have decreased the sensitivity for changes due to sleep pressure at the level of FNC in the brain as the NSP group showed more variance in time awake until their recordings were performed. Also, the NSP and HSP group were recorded at different times of day, thus potentially influenced by circadian differences (Blautzik et al. 2013), though due to the variability in the time of the recordings in the NSP group, this influence – if present – should be fairly small.

Another small potential confound was the lack of caffeine abstention in the NSP group. Caffeine intake has a known (and regionally heterogeneous) effect on brain metabolism (Wu et al. 2014; Xu et al. 2015) that could have influenced the differences between the NSP and HSP group. However, plasma caffeine concentration after administration of doses

comparable to two cups of coffee peaks after 0.5 hours after administration and returns to baseline levels after approximately 8 hours (Kaplan et al. 1997). Further, significant differences on EEG theta activity vanish at 10 hours after caffeine administration (Landolt et al. 2004). Out of 17 subjects in the NSP group, 7 reported caffeine intake prior to the study. In all of these subjects, the time of caffeine consumption was at least 3 hours before the first measurement. Therefore, if influences were still present, they should have been varying and non-systematic in the NSP group. Due to the demanding design of the studies, an approach with more thorough control of the NSP group was not feasible.

Further, our results were limited by the absence of EEG recordings in the NSP group during the CBF session. We cannot rule out minor intrusions of sleep in this group during the CBF recordings. However, as these subjects were supposedly well-rested (which was reflected both by their Epworth sleepiness scores – a measure of daytime sleepiness – and the assessment of their acute sleepiness with the Karolinska sleepiness scale; see Tab. 1), were recorded throughout the daytime, did not fall asleep during the preceding BOLD session and after the recording did not report any sleep intrusions, we are confident that this potential confound is small.

Another limitation stems from the differences regarding the eyes open or closed conditions in the two recording protocols, between the BOLD (2 min EO, 2 min EC, 2 min EO, 2 min EC; Fig. 1) and CBF sessions (HSP: 10 min EO, NSP: 5 min EO, 5 min EC; Fig. 1). Although studies have reported differences in functional connectivity with respect to voluntary eye movements in sleep-deprived subjects (Ong et al. 2015) and vigilance fluctuations (Wang et al. 2016), the reported functional connectivity changes were distinctly different from our observations. Also, since the pattern of cued EO/EC was added as a covariate of no interest in our FNC analysis, this confound should be minimal.

As we had a sex bias (some females only in the NSP group), we performed an additional analysis with the covariate ‘sex’ to address this unbalanced distribution as good as possible. This covariate influenced the FNC due to ‘time awake’. However, the overall ratio of females to males was roughly 1:2, therefore, males dominated. Also, all females were awake for a shorter time, and thus had a lower sleep pressure. This bias might lead to shared variance of ‘time awake’ and ‘sex’ (though no interaction effect of ‘sex’ and ‘time awake’ was observed regarding FNC), and thereby could influence the FNC results. As Online Resource 1 Figure S4 shows, the pattern of FNC due to time awake stayed similar when taking ‘sex’ into account. As in general sex differences in FNC were relatively sparse or even absent in large-cohort studies (Weissman-Fogel et al. 2010; Allen et al. 2011a; Filippi et al. 2013; Hjelmervik et al. 2014) we think a pure sex influence is rather unlikely and therefore negligible.

Finally, an advantage of our study is the careful selection of subjects. We took care to have groups not differing in their corrected mid sleep time on free days (assessed by the MCTQ), i.e. to have a similar chonotype, while presenting significant differences in their subjective sleepiness and the time awake at recordings. Further, unlike most studies investigating sleep restriction or deprivation, we combined most of our fMRI measurements with EEG

recordings, to objectively determine whether subjects were able to maintain wakefulness especially while under high sleep pressure.

Conclusions

We could demonstrate that while RSNs themselves basically do not change their topography in response to increased sleep pressure, the FNC between specific network components changes in a distinctive pattern. This specific change in FNC could potentially reflect both compensatory mechanisms to fight sleep as well a first decrease of consciousness in the form of becoming drowsy. FNC of RSNs with components of the DMN revealed positive effects as a function of time awake and negative effects with all other but the DMN networks (Fig. 4), hinting at a change of the temporal fluctuations of some RSNs in the brain towards the fluctuation pattern of the DMN which might influence information transfer of these networks as a consequence both of increased drowsiness as well as decreased consciousness.

Additionally, we demonstrated that CBF and pBOLD signals are suited to derive ICA-based RSNs and that differences between CBF and BOLD measures were rather small, both with respect to the spatial intensity of the RSNs as well as their between network connectivity (i.e. FNC). Therefore, we could verify that CBF signals are indeed a viable option for these types of analyses. As we find the same networks with different modalities, a strong biological substrate must be underlying these resting state networks.

Author Contributions

L.T., A.S., D.B., R.O.T. and P.A. designed the experiment. L.T. and A.S. performed the experiment. L.T., J.B. and P.A. analyzed the data. L.T. and P.A. wrote the manuscript. All authors approved the final version.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This work was supported by the Swiss National Science Foundation Sinergia grant #136249.

Figure Legends

Figure 1: Study protocols of the normal sleep pressure (NSP; top) and high sleep pressure (HSP; bottom) group. NSP participants were recorded throughout the day (range: 14:25 to 20:53 for BOLD, 15:46 to 22:18 for CBF) after approximately 8 hours of sleep at home. Average time awake at resting state recordings (RSin: BOLD resting state; RSasl: ASL resting state) was 10 hours. Participants in the HSP group had a sleep opportunity of 4 h in the laboratory (23:30 to 03:30) and had to stay awake approximately 19 hours. RSin recordings started on average at 22:21 and RSasl at 23:31. In between BOLD and CBF resting state recordings, participants executed different tasks (NSP: modified Sternberg

working memory task (SB; Michels et al. 2010), modified Eriksen Flanker task (Flanker; Kopp et al. 1996; Bunge et al. 2002; Iannaccone et al. 2015), Stop signal task (Stop; Rubia et al. 2003); HSP: modified Sternberg working memory task (SB; Michels et al. 2010) and had a break. RSout: EEG resting state and calibration recording outside of the scanner. For further details see Tab. 1. EO: eyes open, EC: eyes close, DTI: diffusion tensor imaging, T1: anatomical T1-weighted image.

Figure 2: Spatial map intensities (SMs) of the 17 ICs identified as RSNs. These ICs form seven RSN groups (Tab. 2). SMs are plotted as t-statistics, thresholded according to Allen et al. (2011a) and displayed with the three most informative slices. RSNs were grouped based on their anatomical and functional properties and include subcortical (SUB), auditory (AUD), sensorimotor (SENS), visual (VIS), default mode (DMN), attentional (ATT) and cerebellar (CEREB) networks.

Figure 3: Mean functional network connectivity (FNC) matrix. Pairwise partial correlations between ICs after correction for movements, eyes open/closed and sleep scores are illustrated. R-values were Fisher z-transformed and averaged across subjects, then back-transformed for display.

Figure 4: 'Time awake' effects on the mean FNC matrix. Univariate test results showing the effect of 'time awake' on FNC. Illustrated are the significant $-\text{sign}(t)\log_{10}(p)$ values, meaning that the significant positive or negative effects are displayed on a logarithmic scale. Floating-point numbers on the colorbar indicate the FDR-corrected threshold ($\alpha = 0.05$). Warm colors indicate a positive effect on FNC with longer time awake, while cold colors indicate a negative effect on FNC with longer time awake. Arrows illustrate the direction of the effect: positive effects can be caused by increased positive correlations, decreased negative correlations and switches of negative to positive correlations, while negative effects can be caused by decreased negative correlations or switches from positive to negative correlations (see Online Resource 1 Figure S3 for a detailed interpretation).

Table Legends

Table 1: Participant demographics and behavioral measures. Body mass index (BMI) derived from self-reported weight and height measures. Mean wakeup time derived from the Munich chorotype questionnaire (MCTQ) information in the NSP group and experimental recording time in the HSP group. Time awake at the recording session derived from wakeup time and actual time of start of the recording. ESS: Epworth sleepiness scale (range 0 – 24, 0: lowest possible level of daytime sleepiness, above 10: excessive daytime sleepiness). MCTQ values represent mid sleep point on free days, corrected for age, sex and sleep debt. KSS: Karolinska sleepiness scale (range 1 – 9, 1: extremely alert, 9: extremely sleepy-fighting sleep). Values are shown as mean (standard deviation). *All p-values derived from Mann-Whitney-U test. n.s.: not significant; n.a.: not applicable

Table 2: RSN groups and modality effects on their detection sensitivity. Seventeen ICs were identified forming seven RSN groups (Fig. 2). ICs were classified into RSN groups and assigned to the ICs reported by Allen et al. (2011a) to which they were spatially best

correlated. Coordinates of their peak activations are provided in MNI space [mm]. Component size was derived by a non-overlapping winner-takes-all approach on the t-maps (Fig. 2). Modality effects per RSN group are given as percentage of the total number of voxels. CBF>BOLD indicates higher detection sensitivity of CBF than BOLD and accordingly for other comparisons.

Tables

Table 1: Participant demographics and behavioral measures.

Variables	**Normal sleep pressure (NSP) group**	**High sleep pressure (HSP) group**	**P value***
Age	23.9 (2.3) years	22.4 (2.5) years	n.s.
Gender	7 m, 10 f	17 m	n.a.
BMI	21.8 (2.2) kg/m^2	23.3 (2.3)	n.s.
Wake up time	6:52 (50 min)	3:30 (0 min) kg/m^2	$p < 0.0001$
Time awake at BOLD session	9.8 (2.3) h	18.9 (0.5) h	$p < 0.0001$
Time awake at CBF/pBOLD session	11.2 (2.3) h	20 (0.4) h	$p < 0.0001$
ESS	5.2 (2.8)	4.5 (2)	n.s.
MCTQ (mid sleep free days corrected)	4:32 (1:44)	4:06 (1:07)	n.s.
KSS	4.4 (2.1)	6.7 (1.3)	$p < 0.002$

*Mann-Whitney-U-Test

Table 2: RSN groups and modality effects on their detection sensitivity.

Network groups	**Allen et al. (2011a)**	**IC**	**Peak voxel**			**Size (Nr of voxels)**	**Modality difference CBF vs BOLD (%) CBF>BOLD/ CBF<BOLD**	**Modality difference pBOLD vs BOLD (%) pBOLD>BOLD/ pBOLD<BOLD**	**Modality difference CBF vs pBOLD (%) CBF>pBOLD/ CBF<pBOLD**
			x	**y**	**z**				
Subcortical Network (SUB)	(IC 21: basal ganglia)	IC 7	-6	-19	7	2514	29.3 %/0.72 %	25.8 %/0.20 %	0.36 %/0.40 %
Auditory Network (AUD)	IC 17	IC 4	-60	-40	7	3377	7.43 %/0.36 %	1.01 %/0.06 %	0.44 %/0.09 %
Sensorimotor Networks (SENS)	IC 7 IC 23 IC 24 IC 38 IC 56 IC29	IC 5 IC 12 IC 17 IC 21	-57 60 -27 3	-7 -34 -1 -22	22 34 55 55	2657 1470 2377 3887 Total: 10391	20.9 %/2.10 %	6.84 %/0.60 %	0.82 %/0.72 %
Visual Networks (VIS)	IC 46 IC64 IC 67 IC48 IC 39 IC 59	IC 15 IC 2 IC 18	24 12 42	-91 -73 -76	-2 4 1	2292 3157 2672 Total: 8121	5.91 %/1.74 %	0.80 %/0.06 %	1.15 %/0.37 %
Default Mode Networks (DMN)	IC 50 IC 53 IC 25 IC 68	 IC 20 IC 23 IC 22 IC 10	 3 9 -54 -30	 -49 -58 -61 47	 31 19 28 34	 2149 873 3147 1308 Total: 7477	8.88 %/1.00 %	2.41 %/0.35 %	0.37 %/0.12 %
Attentional Networks (ATT)	IC 34 IC 60 IC52 IC 72 IC 71 IC 55	IC 24 IC 14 IC 8	-45 39 0	-49 -67 11	43 43 55	2712 3036 1608 Total: 7356	7.64 %/1.17 %	3.90 %/0.24 %	0.52 %/0.07 %
Cerebellar Network (CEREB)		IC 16	15	-67	-26	3346	3.14 %/0.4 5%	0.90%/0.18%	÷/÷

IC: Independent Component

References

Aguirre GK, Detre JA, Zarahn E, Alsop DC (2002) Experimental Design and the Relative Sensitivity of BOLD and Perfusion fMRI Neuroimage 15:488-500

Åkerstedt T, Gillberg M (1990) Subjective and objective sleepiness in the active individual The International Journal of Neuroscience 52:29-37

Allen EA et al. (2011a) A baseline for the multivariate comparison of resting-state networks Frontiers in Systems Neuroscience 5:1-23

Allen EA, Erhardt EB, Yonghua W, Eichele T, Calhoun VD (2011b) Capturing inter-subject variability with group independent component analysis of fMRI data: A simulation study Neuroimage 59:4141-4159 doi:10.1016/j.neuroimage.2011.10.010

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

Aslan S, Xu F, Wang PL, Uh J, Yezhuvath US, van Osch M, Lu H (2010) Estimation of Labeling Efficiency in Pseudocontinuous Arterial Spin Labeling Magn Reson Med 63:765-771

Banks S, Dinges DF (2007) Behavioral and Physiological Consequences of Sleep Restriction Journal of Clinical Sleep Medicine 3:519-528

Basner M, Rao H, Goel N, Dinges DF (2013) Sleep deprivation and neurobehavioral dynamics Curr Opin Neurobiol 23:854-863

Beckmann CF, DeLuca M, Devlin JT, Smith SM (2005) Investigations into resting-state connectivity using independent component analysis Philos Trans R Soc Lond B Biol Sci 360:1001-1013

Berger RJ, Oswald I (1962) Effects of sleep deprivation on behaviour, subsequent sleep, and dreaming The British Journal of Psychiatry 108:457-465

Biswal B, Yetkin FZ, 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

Blautzik J et al. (2013) Classifying fMRI-derived resting-state connectivity patterns according to their daily rhythmicity Neuroimage 71:298-306

Braun AR et al. (1997) Regional cerebral blood flow throughout the sleep--wake cycle An H2(15)O PET study Brain 120:1173-1197

Buckner RL, Andrews-Hanna JR, Schacter DL (2008) The Brain's Default Network: Anatomy, Function, and Relevance to Disease Ann N Y Acad Sci 1124:1-38

Bunge S, Hazeltine E, Scanlon M, Rosen A, Gabrieli J (2002) Dissociable contributions of prefrontal and parietal cortices to response selection Neuroimage 17:1562-1571 doi:http://dx.doi.org/10.1006/nimg.2002.1252

Buxton RB (2002) Introduction to Functional Magnetic Resonance Imaging: Principles and Techniques. Cambridge University Press,

Buzsáki G, Logothetis N, Singer W (2013) Scaling brain size, keeping timing: evolutionary preservation of brain rhythms Neuron 80:751-764 doi:10.1016/j.neuron.2013.10.002

Calhoun VD, Adali T, Pearlson GD, Pekar JJ (2001) A Method for Making Group Inferences from Functional MRI Data Using Independent Component Analysis Hum Brain Mapp 14:140-151

Chee MWL, Choo W (2004) Functional imaging of working memory after 24 hr of total sleep deprivation J Neurosci 24:4560–4567

Chee MWL, Chuah L, Venkatraman V, Chan W, Philip P, Dinges D (2006) Functional imaging of working memory following normal sleep and after 24 and 35 h of sleep

deprivation: correlations of fronto-parietal activation with performance Neuroimage 31:419–428

Chee MWL, Tan J, Parimal S, Zagorodnov V (2010) Sleep deprivation and its effects on object-selective attention Neuroimage 49:1903-1910 doi:10.1016/j.neuroimage.2009.08.067

Chee MWL, Tan J, Zheng H, Parimal S, Weissmann D, Zagorodnov V, Dinges DF (2008) Lapsing during sleep deprivation is associated with distributed changes in brain activation J Neurosci 28:5519-5528 doi:10.1523/JNEUROSCI.0733-08.2008

Chee MWL, Tan JC (2010) Lapsing when sleep deprived: Neural activation characteristics of resistant and vulnerable individuals Neuroimage 51:835-843

Chen JJ, Jann K, Wang DJJ (2015) Characterizing Resting-State Brain Function using Arterial-Spin Labeling Brain Connectivity 5:527-542

Choo W-C, Lee W-W, Venkatraman V, Sheu F-S, Chee MWL (2005) Dissociation of cortical regions modulated by both working memory load and sleep deprivation and by sleep deprivation alone Neuroimage 25:579-587

Czisch M, Wehrle R, Harsay H, Wetter TC, Holsboer F, Sämann PG, Drummond SPA (2012) On the need of objective vigilance monitoring: effects of sleep loss on target detection and task-negative activity using combined EEG/fMRI Front Neurol 3:67

Damoiseaux JS, Rombouts SARB, Barkhof F, Scheltens P, Stam CJ, Smith SM, Beckmann CF (2006) Consistent resting-state networks across healthy subjects Proc Natl Acad Sci U S A 103:13848-13853

De Havas JA, Parimal S, Soon CS, Chee MWL (2012) Sleep deprivation reduces default mode network connectivity and anti-correlation during rest and task performance Neuroimage 59:1745-1751

De Luca M, Beckmann CF, De Stefano N, Matthews PM, Smith SM (2006) fMRI resting state networks define distinct modes of long-distance interactions in the human brain Neuroimage 29:1359-1367

Dehaene S, Sergent C, Changeaux J-P (2003) A neuronal network model linking subjective reports and objective physiological data during conscious perception Proc Natl Acad Sci U S A 100:8520-8525

Deibler AR, Pollock JM, Kraft RA, Tan H, Burdette JH, Maldjian JA (2008) Arterial Spin-Labeling in Routine Clinical Practice, Part 1: Technique and Artifacts AJNR Am J Neuroradiol 29:1228-1234

Dinges DF et al. (1997) Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4-5 hours per night Sleep 20:267-277

Drummond S, Bischoff-Grethe A, Dinges D, Ayalon L, Mednick S, Meloy M (2005) The neural basis of the psychomotor vigilance task Sleep 28:1059–1068

Drummond S, Gillin J, Brown G (2001) Increased cerebral response during a divided attention task following sleep deprivation J Sleep Res 10:85–92

Drummond SPA, Brown GG, Salamat JS, Gillin JC (2004) Increasing Task Difficulty Facilitates the Cerebral Compensatory Response to Total Sleep Deprivation Sleep 27:445-451

Erhardt EB, Rachakonda S, Bedrick E, Allen EA, Adali T, Calhoun VD (2011) Comparison of multi-subject ICA methods for analysis of fMRI data Hum Brain Mapp 32:2075-2095 doi:10.1002/hbm.21170

Fan J, McCandliss BD, Fossella J, Flombaum JI, Posner MI (2005) The Activation of Attention Networks Neuroimage 26:471-479

Filippi M, Valsasina P, Misci P, Falini A, Comi G, Rocca MA (2013) The Organization of Intrinsic Brain Activity Differs Between Genders: A Resting-State fMRI Study in a

Large Cohort of Young Healthy Subjects Hum Brain Mapp 34:1330-1343 doi:10.1002/hbm.21514

Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME (2005) The human brain is intrinsically organized into dynamic, anticorrelated functional networks Proc Natl Acad Sci U S A 102:9673-9678

Franco A, Mannell M, Calhoun VD, Mayer AR (2013) Impact of Analysis Methods on the Reproducibility and Reliability of Resting-State Networks Brain Connectivity 3:363-374 doi:10.1089/brain.2012.0134

Heine L et al. (2012) Resting state networks and consciousness Alterations of multiple resting state network connectivity in physiological, pharmacological, and pathological consciousness states Frontiers in Psychology 3

Hjelmervik H, Hausmann M, Osnes B, Westerhausen R, Specht K (2014) Resting States Are Resting Traits - An fMRI Study of Sex Differences and Menstrual Cycle Effects in Resting State Cognitive Control Networks PloS One 9:e103492 doi:10.1371/journal.pone.0103492

Hockey GRJ, Wastell DG, Sauer J (1998) Effects of sleep deprivation and user interface on complex performance: a multilevel analysis of compensatory control Hum Factors 40:233-253

Horne JA, Pettitt AN (1985) High Incentive Effects on Vigilance Performance during 72 Hours of Total Sleep Deprivation Acta Psychol (Amst) 58:123-139

Horovitz SG, Braun AR, Carr WS, Picchioni D, Balkin TJ, Fukunaga M, Duyn JH (2009) Decoupling of the brain}s default mode network during deep sleep Proc Natl Acad Sci U S A 106:11376-11381

Horovitz SG, Fukunaga M, de Zwart JA, van Gelderen P, Fulton SC, Balkin TJ, Duyn JH (2008) Low Frequency BOLD Fluctuations During Resting Wakefulness and Light Sleep: A Simultaneous EEG-fMRI Study Hum Brain Mapp 29:671-682

Iannaccone R, Hauser T, Staempfli P, Walitza S, Brandeis D, Brem S (2015) Conflict monitoring and error processing: new insights from simultaneous EEG-fMRI Neuroimage 105:395-407 doi:http://dx.doi.org/10.1016/j.neuroimage.2014.10.028

Iber C, Ancoli-Israel S, Chesson Jr AL, Quan SF (2007) The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications, 1st edn. American Academy of Sleep Medicine, 1 Westbrook Corporate Center, Suite 920, Westchester, IL 60154, USA.

Jackson ML, Gunzelmann G, Whitney P, Hinson JM, Belenky G, Rabat A, Van Dongen HPA (2013) Deconstructing and Reconstructing Cognitive Performance in Sleep Deprivation Sleep Medicine Reviews 17:215-225

Jackson ML et al. (2011) The effect of sleep deprivation on BOLD activity elicited by a divided attention task Brain Imaging Behav 5:97-108 doi:10.1007/s11682-011-9115-6

Jann K, Gee DG, Kilroy E, Schwab S, Smith RX, Cannon TD, Wang DJJ (2015) Functional connectivity in BOLD and CBF data: Similarity and reliability of resting brain networks Neuroimage 106:111-122

Jann K, Orosz A, Dierks T, Wang DJJ, Wiest R, Federspiel A (2013) Quantification of Network Perfusion in ASL Cerebral Blood Flow Data with Seed Based and ICA Approaches Brain Topogr 26:569-580

Johns MW (1991) A New Method for Measuring Daytime Sleepiness: The Epworth Sleepiness Scale Sleep 14:540-545

Kaplan GB, Greenblatt DJ, Ehrenberg BL, Goddard JE, Cotreau MM, Harmatz JS, Shader RI (1997) Dose-Dependent Pharmacokinetics and Psychomotor Effects of Caffeine in Humans Pharmcokinetics and Pharmacodynamics 37:693-703

Kaufmann T et al. (2016) The brain functional connectome is robustly altered by lack of sleep Neuroimage 127:324-332

Kong D, Soon C, Chee MWL (2012) Functional imaging correlates of impaired distractor suppression following sleep deprivation Neuroimage 61:50-55

Kopp B, Rist F, Mattler U (1996) N200 in the flanker task as a neurobehavioral tool for investigating executive control Psychophysiology 33:282-294

Laird AR et al. (2011) Behavioral Interpretations of Intrinsic Connectivity Networks J Cogn Neurosci 23:4022-4037

Landolt H-P, Rétey JV, Tönz K, Gottselig JM, Khatami R, Isabelle B, Achermann P (2004) Caffeine Attenuates Waking and Sleep Electroencephalographic Markers of Sleep Homeostasis in Humans Neuropsychopharmacology 29:1933-1939

Larson-Prior LJ, Zempel JM, Nolan TS, Prior FW, Snyder AZ, Raichle ME (2009) Cortical network functional connectivity in the descent to sleep Proc Natl Acad Sci U S A 106:4489-4494

Li YO, Adali T, Calhoun VD (2007) Estimating the number of independent components for functional magnetic resonance imaging data Hum Brain Mapp 28:1251-1266

Lim J, Choo W, Chee MWL (2007) Reproducibility of changes in behaviour and fMRI activation associated with sleep deprivation in a working memory task Sleep 30:61–70

Lim J, Tan J, Parimal S, Dinges DF, Chee MWL (2010) Sleep deprivation impairs object-selective attention: a view from the ventral visual cortex PloS One 5:e9087

Liu TT, Wong EC (2005) A signal processing model for arterial spin labeling functional MRI Neuroimage 24:207-215

Logothetis N, Pauls J, Augath M, Trinath T, Oeltermann A (2001) Neurophysiological investigation of the basis of the fMRI signal Nature 412:150-157 doi:10.1038/35084005

Ma N, Dinges DF, Basner M, Rao H (2015) How Acute Total Sleep Loss Affects the Attending Brain: A Meta-Analysis of Neuroimaging Studies Sleep 38:233-240

Magri C, Schridde U, Murayama Y, Panzeri S, Logothetis N (2012) The Amplitude and Timing of the BOLD Signal Reflects the Relationship between Local Field Potential Power at Different Frequencies J Neurosci 32:1395-1407 doi:10.1523/JNEUROSCI.3985-11.2012

Mander B et al. (2008) Sleep deprivation alters functioning within the neural network underlying the covert orienting of attention Brain Res 1217:148-156 doi:10.1016/j.brainres.2008.04.030

Mantini D, Perrucci MG, Cugini S, Ferretti A, Romani GL, Del Gratta C (2007) Complete artifact removal for EEG recorded during continuous fMRI using independent component analysis Neuroimage 34:598-607

Mason MF, Norton MI, Van Horn JD, Wegner DM, Grafton ST, Macrae CN (2007) Wandering minds: the default network and stimulus-independent thought Science 315:393-395

Michels L, Bucher K, Lüchinger R, Klaver P, Martin E, Jeanmonod D, Brandeis D (2010) Simultaneous EEG-fMRI during a working memory task: modulations in low and high frequency bands PloS One 5:e10298

Mu Q et al. (2005) Decreased cortical response to verbal working memory following sleep deprivation Sleep 28:55–67

Muto V et al. (2012) Influence of acute sleep loss on the neural correlates of alerting, orientating and executive attention components J Sleep Res 21:648-658 doi:10.1111/j.1365-2869.2012.01020.x

Nuwer MR et al. (1998) IFCN Standards - IFCN standards for digital recording of clinical EEG Electroencephalogr Clin Neurophysiol 106:259-261 doi:10.1016/S0013-4694(97)00106-5

Ong J, Kong D, Chia T, Tandi J, Yeo BTT, Chee MWL (2015) Co-activated yet disconnected—Neural correlates of eye closures when trying to stay awake Neuroimage 118 doi:10.1016/j.neuroimage.2015.03.085

Portas CM, Howseman AM, Josephs O, Turner R, Frith CD (1998) A Specific Role for the Thalamus in Mediating the Interaction of Attention and Arousal in Humans The Journal of Neuroscience 18:8979-8989

Poudel GR, Innes CRH, Jones RD (2012) Cerebral Perfusion Differences Between Drowsy and Nondrowsy Individuals After Acute Sleep Restriction Sleep 35:1085-1096

Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE (2012) Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion Neuroimage 59:2142-2154

Raichle ME (2011) The Restless Brain Brain Connectivity 1:3-12

Rechtschaffen A, Kales A (1968) A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects. U. S. Department of Health, Education. and Welfare, Public Health Service - National Institutes of Health, National Institute of Neurological Diseases and Blindness, Neurological Information Network, Bethesda, Maryland 20014

Roenneberg T, Wirz-Justice A, Merrow M (2003) Life between Clocks: Daily Temporal Patterns of Human Chronotypes J Biol Rhythms 18:80-90

Rubia K, Smith A, Brammer M, Taylor E (2003) Right inferior prefrontal cortex mediates response inhibition while mesial prefrontal cortex is responsible for error detection Neuroimage 20:351-358

Sämann PG, Tully C, Spoormaker VI, Wetter TC, Holsboer F, Wehrle R, Czisch M (2010) Increased sleep pressure reduces resting state functional connectivity Magnetic Resonance Materials in Physics, Biology and Medicine 23:375-389

Sämann PG et al. (2011) Development of the Brain's Default Mode Network from Wakefulness to Slow Wave Sleep Cereb Cortex 21:2082-2093

Smith SM et al. (2009) Correspondence of the brain}s functional architecture during activation and rest Proc Natl Acad Sci U S A 106:13040-13045

Sternberg S (1966) High-Speed Scanning in Human Memory Science 153:652-654

Tomasi D et al. (2009) Impairment of attentional networks after 1 night of sleep deprivation Cereb Cortex 19:233–240 doi:10.1093/cercor/bhn073

Wang C, Ong J, Patanaik A, Zhou J, Chee M (2016) Spontaneous eyelid closures link vigilance fluctuation with fMRI dynamic connectivity states Proc Natl Acad Sci U S A 113:9653-9658

Webb WB, Levy CM (1984) Effects of spaced and repeated total sleep deprivation Ergon 27:45-58

Weissman-Fogel I, Moayedi M, Taylor KS, Pope G, Davis KD (2010) Cognitive and Default-Mode Resting State Networks: Do Male and Female Brains "Rest" Differently? Hum Brain Mapp 31:1713-1726 doi:10.1002/hbm.20968

Wilkinson RT (1961) Interaction of lack of sleep with knowledge of results, repeated testing and individual differences J Exp Psychol 62:263-271

Williams DS, Detre JA, Leigh JS, Koretsky AP (1992) Magnetic resonance imaging of perfusion using spin inversion of arterial water Proc Natl Acad Sci U S A 89:212-216

Wittmann M, Dinich J, Merrow M, Roenneberg T (2006) Social Jetlag: Misalignment of Biological and Social Time Chronobiol Int 23:497-509

Wong EC, Buxton RB, Frank LR (1997) Implementation of Quantitative Perfusion Imaging Techniques for Functional Brain Mapping using Pulsed Arterial Spin Labeling NMR Biomed 10:237-249

Wu JC et al. (2006) Frontal Lobe Metabolic Decreases with Sleep Deprivation not Totally Reversed by Recovery Sleep Neuropsychopharmacology 31:2783-2792

Wu W-C, Lien S-H, Chang J-H, Yang S-C (2014) Caffeine alters resting-state functional connectivity measured by blood oxygenation level-dependent MRI NMR Biomed 27:444-452

Wu W-C, St Lawrence KS, Licht DJ, Wang DJJ (2010) Quantification Issues in Arterial Spin Labeling Perfusion Magnetic Resonance Imaging Top Magn Reson Imaging 21:65-73

Xu F, Liu P, Pekar JJ, Lu H (2015) Does acute caffeine ingestion alter brain metabolism in young adults? Neuroimage 110:39-47

Yeo BTT, Tandi J, Chee MWL (2015) Functional connectivity during rested wakefulness predicts vulnerability to sleep deprivation Neuroimage 111:147-158

Figure 1

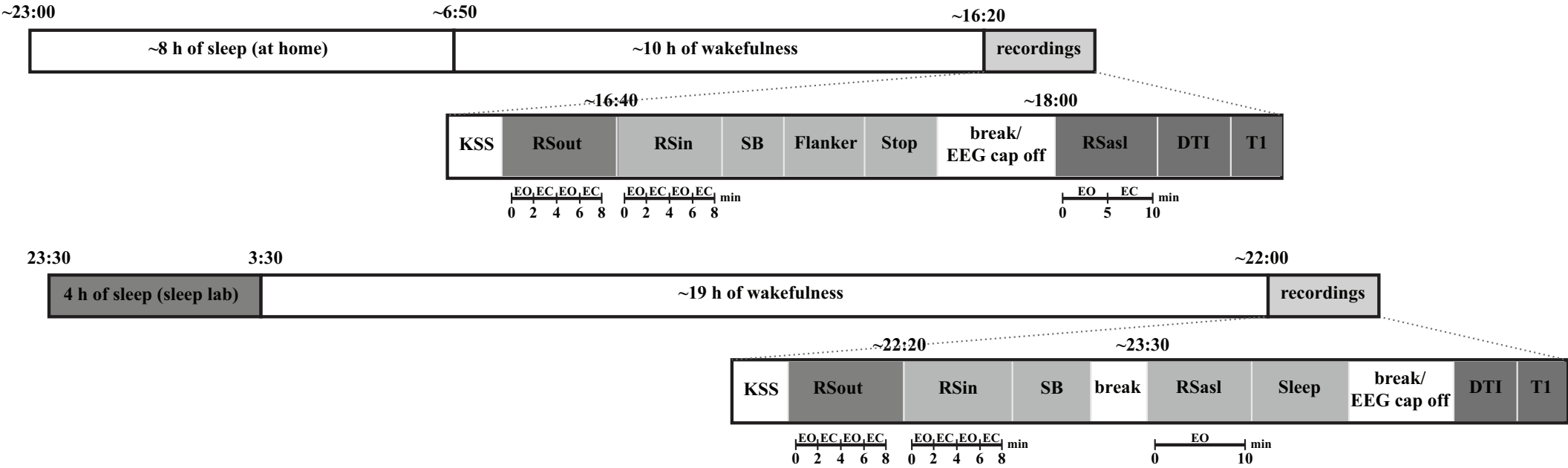

~23:00
~6:50
~16:20
~8 h of sleep (at home)
~10 h of wakefulness
recordings
~16:40
~18:00
KSS
RSout
RSin
SB
Flanker
Stop
break/
EEG cap off
RSasl
DTI
T1
EO EC EO EC
0 2 4 6 8
EO EC EO EC
0 2 4 6 8
min
EO
EC
0 5 10
min
23:30
3:30
~22:00
4 h of sleep (sleep lab)
~19 h of wakefulness
recordings
~22:20
~23:30
KSS
RSout
RSin
SB
break
RSasl
Sleep
break/
EEG cap off
DTI
T1
EO EC EO EC
0 2 4 6 8
EO EC EO EC
0 2 4 6 8
min
EO
0 10
min

Figure 2

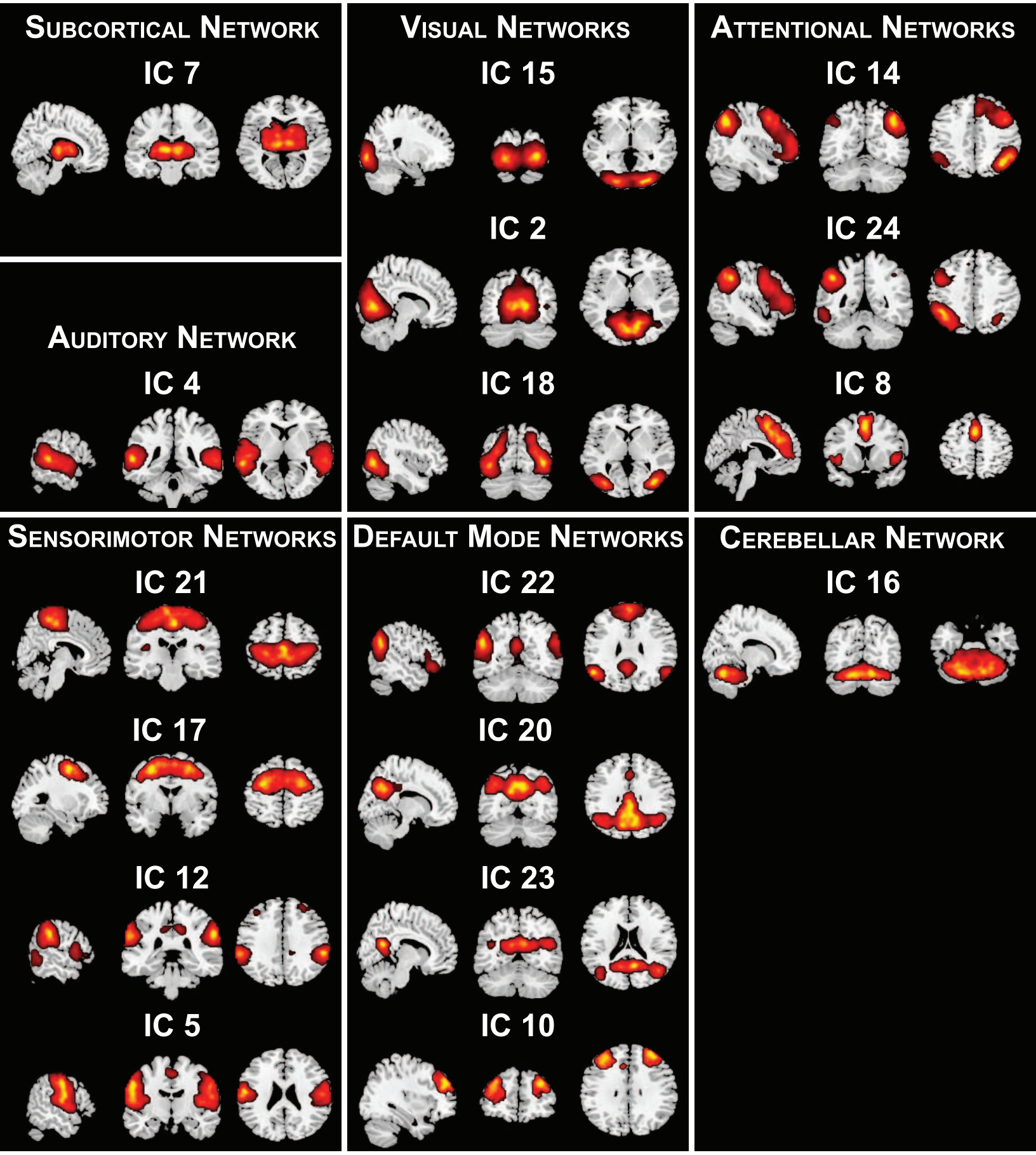

Subcortical Network
IC 7
Visual Networks
IC 15
IC 2
IC 18
Attentional Networks
IC 14
IC 24
IC 8
Auditory Network
IC 4
Sensorimotor Networks
IC 21
IC 17
IC 12
IC 5
Default Mode Networks
IC 22
IC 20
IC 23
IC 10
Cerebellar Network
IC 16

Figure 3

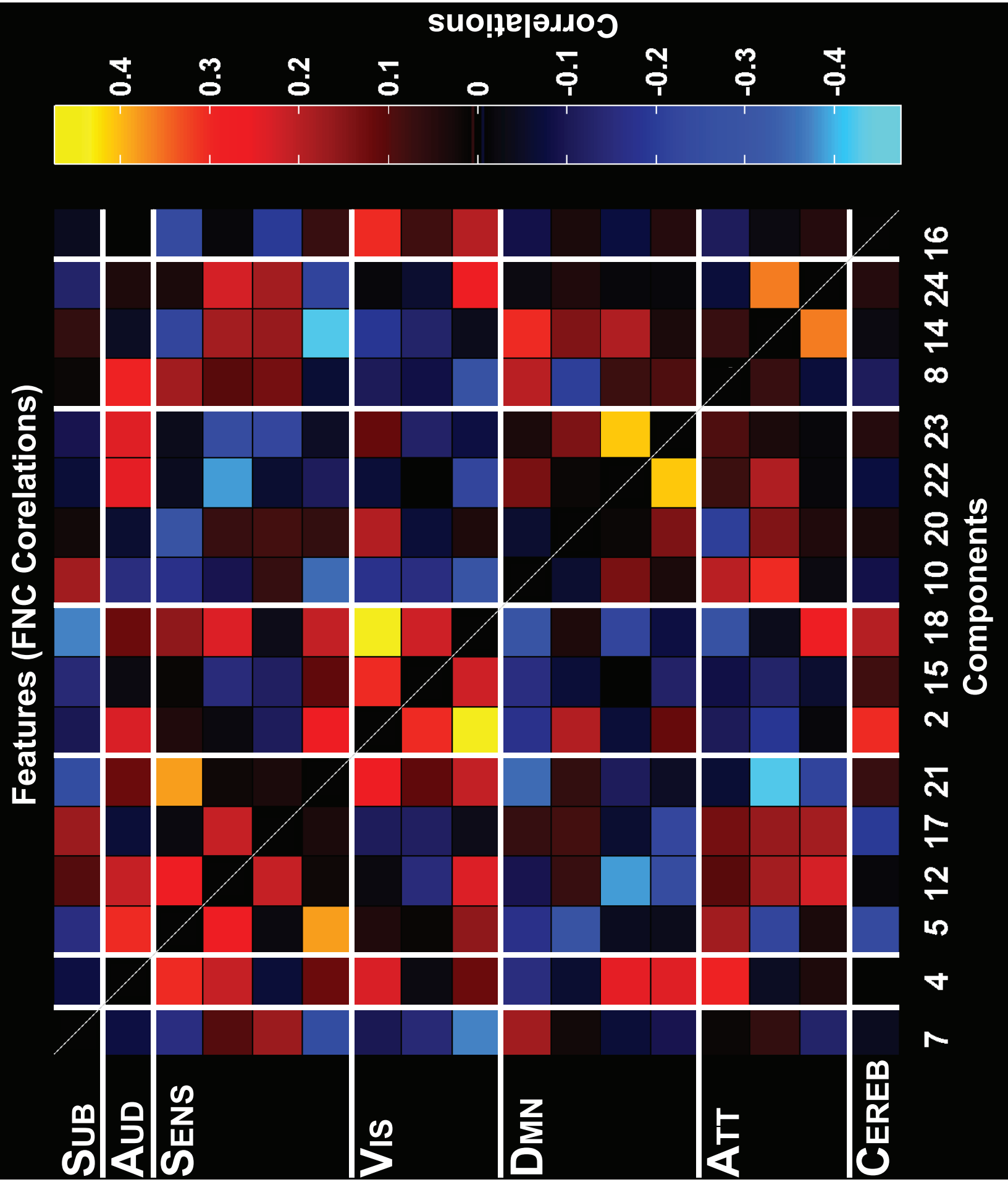
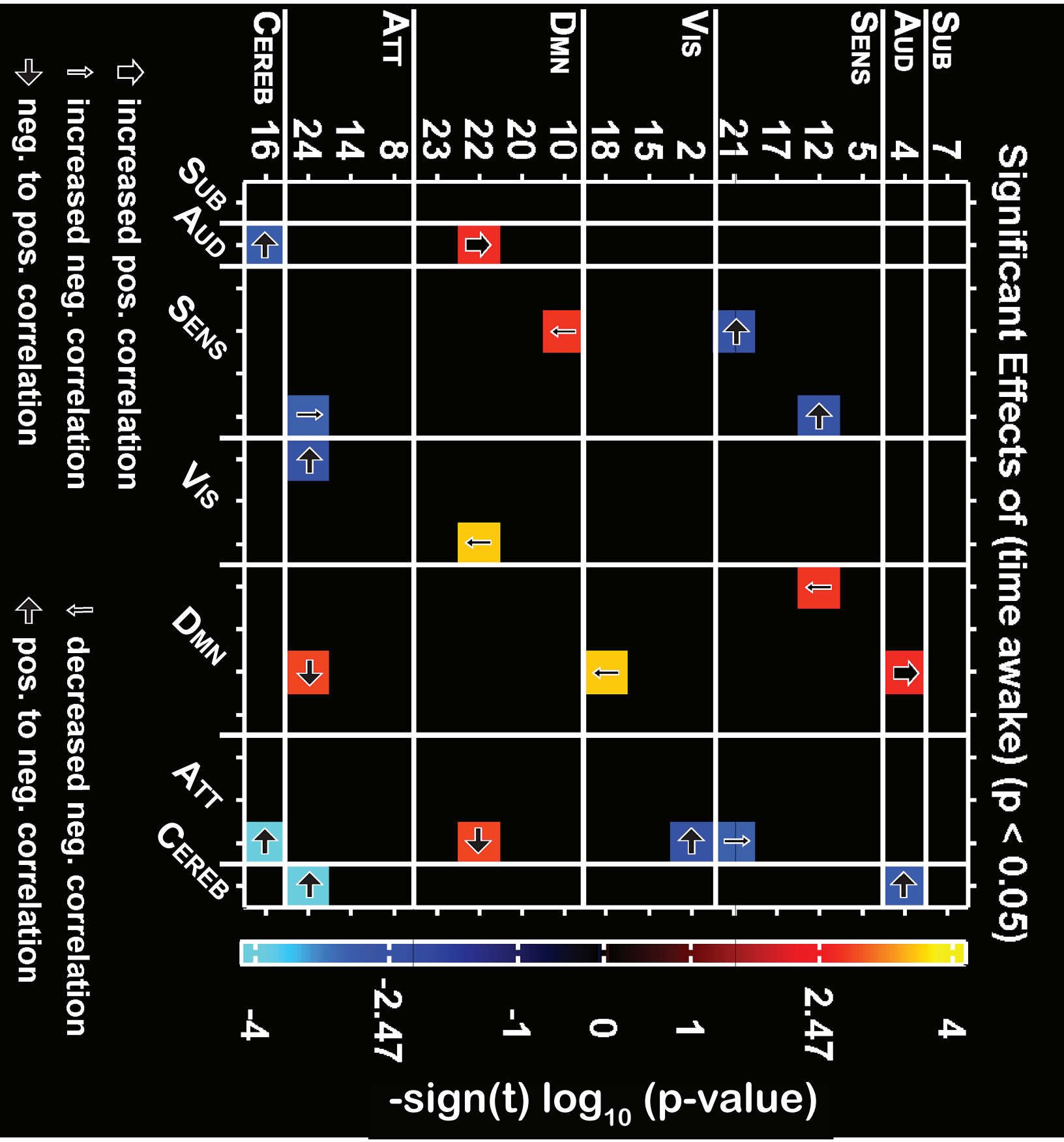

Correlations
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
Features (FNC Corelations)
SUB
AUD
SENS
VIS
DMN
ATT
CEREB
7 4 5 12 17 21 2 15 18 10 20 22 23 8 14 24 16
Components

Figure 4


Significant Effects of (time awake) (p < 0.05)
Sub 7
Aud 4
Sens 5 12 17 21
Vis 2 15 18
Dmn 10 20 22 23
Att 8 14 24
Cereb 16
Sub
Aud
Sens
Vis
Dmn
Att
Cereb
-sign(t) log10 (p-value)
4
2.47
1
0
-1
-2.47
-4
⇨ increased pos. correlation
⇧ increased neg. correlation
⇩ neg. to pos. correlation
⇦ decreased neg. correlation
⇧ pos. to neg. correlation