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Cerebral blood flow changes after a day of wake, sleep, and sleep deprivation

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ABSTRACT

Elucidating the neurobiological effects of sleep and wake is an important goal of the neurosciences. Whether and how human cerebral blood flow (CBF) changes during the sleep-wake cycle remain to be clarified. Based on the synaptic homeostasis hypothesis of sleep and wake, we hypothesized that a day of wake and a night of sleep deprivation would be associated with gray matter resting CBF (rCBF) increases and that sleep would be associated with rCBF decreases. Thirty-eight healthy adult males (age 22.1 ± 2.5 years) underwent arterial spin labeling perfusion magnetic resonance imaging at three time points: in the morning after a regular night's sleep, the evening of the same day, and the next morning, either after total sleep deprivation ($n=19$) or a night of sleep ($n=19$). All analyses were adjusted for hematocrit and head motion. rCBF increased from morning to evening and decreased after a night of sleep. These effects were most prominent in bilateral hippocampus, amygdala, thalamus, and in the occipital and sensorimotor cortices. Group x time interaction analyses for evening versus next morning revealed significant interaction in bilateral lateral and medial occipital cortices and in bilateral insula, driven by rCBF increases in the sleep deprived individuals and decreases in the sleepers, respectively. Furthermore, group x time interaction analyses for first morning versus next morning showed significant effects in medial and lateral occipital cortices, in anterior cingulate gyrus, and in the insula, in both hemispheres. These effects were mainly driven by CBF increases from TP1 to TP3 in the sleep deprived individuals. There were no associations between the rCBF changes and sleep characteristics, vigilant attention, or subjective sleepiness that remained significant after adjustments for multiple analyses. Altogether,

these results encourage future studies to clarify mechanisms underlying sleep-related rCBF changes.

1. INTRODUCTION

Sleep is an enigmatic, evolutionarily conserved process required for human health and functioning¹⁻³. Sleep disturbances are common in the general population⁴⁻⁷ and associated with impaired cognitive performance, lowered work productivity, elevated risk of accidents, and increased prevalence of chronic somatic illnesses^{2,8,9}. Sleep-wake cycle disturbances are also frequent features of neuropsychiatric and neurological disorders^{10,11} and sleep deprivation can have rapid antidepressive effects in mood disorders^{12,13}. The incomplete understanding of sleep and wake processes is one important factor underlying the vast detrimental health effects and societal costs of sleep insufficiency in modern society^{2,8,9}. Elucidating the neurobiological effects of waking and sleep is therefore an important goal in basic and clinical neurosciences.

The dominating theory of sleep regulation is the two-process model^{14,15}. This model distinguishes between circadian and homeostatic mechanisms that together determine the timing of sleep and wake. The circadian component – process C – is a near-24 h self-sustained oscillator influencing the sleep-wake cycle, and is governed by the suprachiasmatic nucleus (SCN) in the anterior hypothalamus¹⁶. The homeostatic component – process S – accumulates exponentially during wakefulness and is discharged during sleep. Although the neural substrates of process S remain to be clarified, the synaptic homeostasis hypothesis posits that process S reflects synaptic strength^{17,18}. This hypothesis is supported by a growing body of animal and human evidence¹⁹⁻²⁵ and suggests that learning and experience during wake promote synaptic strengthening, whereas sleep favors synaptic

downscaling¹⁷.

The majority of the brain's energy is used on synapses and synaptic strengthening increases the metabolic cost^{26,27}. One prediction of the synaptic homeostasis hypothesis is therefore that cerebral metabolic rate (CMR), and, in turn, cerebral blood flow (CBF) of gray matter, should increase after wake and decrease after sleep^{17,28}. Previous studies of CMR and CBF after wake and sleep have reported mixed results; some reports suggested that CBF and CMR fluctuate over the course of a single day²⁹⁻³¹, whereas other studies did not find such variation³². Thus, whether CMR and CBF exhibit sleep-wake cycle-dependent changes remains to be clarified. In the present study, we investigated resting CBF (rCBF) in healthy young male adults after day of wake, sleep, and sleep deprivation using arterial spin labeling (ASL) perfusion magnetic resonance imaging (MRI)³³. We hypothesized that a day of wake and a night of sleep deprivation would be associated with widespread gray matter rCBF increases and that sleep would be associated with rCBF decreases, as predicted by the synaptic homeostasis hypothesis^{17,28}. Secondly, we examined whether rCBF changes after wake and sleep were associated with chronotype, sleep habits, and vigilant attention.

2. MATERIALS AND METHODS

2.1 Ethics statement

Ethical approval was obtained from the Norwegian South East Regional Ethics Committee and all procedures were in line with the declaration of Helsinki. All participants signed informed consent prior to enrolment and received NOK 500 for their participation.

2.2 Descriptive characteristics and study design

Forty right-handed, healthy, adult male volunteers were included. Functional and structural MRI data from overlapping samples were previously investigated³⁴⁻³⁶. We included males only, in order to exclude potential variability due to hormonal cycles. One individual was excluded from the study due to head motion artifacts, and another did not finalize all MRI sessions. The mean age of the final sample (n=38) was 22.1 ± 2.5 years (Table 1). Before study inclusion, all participants were screened for current and previous psychiatric and somatic illnesses. Exclusion criteria were: history of sleep disorders, psychiatric, neurological or chronic somatic disorders, current acute somatic illnesses, use of psychotropic drugs, alcohol or drug use disorders, former head injury with loss of consciousness for more than one minute, and standard MRI exclusion criteria.

The study protocol is summarized in Figure 1A. All participants underwent MRI at the Oslo University Hospital in the morning after a night of regular sleep at home (time point (TP) = 1) and the same evening after approximately 14 hours of waking (TP2). Participants were allowed to leave the hospital between TP1 and TP2, and were instructed not to sleep during

this time. Participants were then assigned either to a sleep group (n=19) or a sleep deprivation group (n=19). The sleep group and the sleep deprivation group were then scanned the next morning (TP3) after a night of sleep at home or after a night of total sleep deprivation, respectively. The sleep deprivation group was supervised at the hospital to ensure they stayed awake. Physical exercise and consumption of alcohol, caffeine and nicotine were prohibited from the night before the study day until study completion. No intake of food or energy-containing fluids was allowed three hours prior to each MRI session.

2.3 Assessment of wake and sleep parameters and vigilant attention

Participants were interviewed about their sleep habits, including their sleep duration on the night, the week, and the month before study participation. Chronotype was measured using Horne-Östberg Morningness-Eveningness (HOME) questionnaire³⁷, whereas general level of daytime sleepiness propensity was assessed using the Epworth Sleepiness Scale (ESS)³⁸. Sleep quality the last month was measured using Pittsburgh Sleep Quality Index (PSQI)³⁹. Subjective sleepiness was assessed at each time point using the Karolinska Sleepiness Scale (KSS)⁴⁰, which is a nine point Likert-type scale ranging from “Extremely alert” (1) to “extremely sleepy” (9). To obtain an objective estimate of vigilant attention at all TPs, the participants performed the attention network task⁴¹ immediately after each MRI acquisition. This task yields reaction time measures in three different conditions by flankering target stimuli with congruent, incongruent or neutral stimuli, thereby altering the difficulty of the task (neutral < congruent < incongruent). As described previously³⁵, we

estimated the intra-individual variation in reaction time, defined as the coefficient of variation in response time, and mean reaction time across the three attention network task flanker conditions.

2.4 Measurement of blood hematocrit

To assess potential fluctuations in longitudinal relaxation rate of blood ($T1_b$), venous blood was drawn from the antecubal vein immediately after each scan, and hematocrit (Hct) was measured using a Sell-Dyn Sapphire Hematology System (Abbott Diagnostics, Illinois, US). The coefficient of variation of the individual analysis system in use was 2.4%. Hct values were used to derive Hct-corrected $T1$ -relaxation times in blood⁴².

2.5 MRI acquisition

MRI was performed on a 3T Achieva scanner (Philips Healthcare, Best, the Netherlands), equipped with an 8-channel head coil (InVivo, Gainesville, FL, US) and foam padding to restrict head motion. Participants were instructed to have their eyes open during all ASL scans. rCBF measurements were performed using a pseudo-continuous ASL⁴³ sequence with a gradient-echo single-shot echo-planar imaging readout with acquisition parameters: matrix = 64x64, field-of-view = 210x210 mm, 22 axial 6 mm slices (no gap), echo time/repetition time (TR) = 11/4400 ms, flip angle = 90 degrees, SENSE factor = 2, 60 control-label pairs, total scan duration = 9 min. Labeling was performed with a train of Hanning-shaped RF pulses – flip angle = 18°, pulse duration = 0.5 ms – with an inter-pulse interval of 1 ms in a balanced gradient scheme⁴⁴, with post-label delay = 1800-2640 ms

(from first to last slice) and labeling duration = 1800 ms. The labeling plane was positioned based on a time-of-flight angiogram. Background suppression was achieved by applying a saturation pulse before labeling and two inversion pulses during the post-label delay. An M0 reference scan was acquired with the same ASL readout without background suppression or labeling (TR=12s, 3 averages). An 1x1x1.2 mm 3D T1-weighted anatomic image was acquired for segmentation and registration purposes.

2.6 ASL image processing

SPM12 (www.fil.ion.ucl.ac.uk/spm/software/spm12) and ExploreASL were used for image processing and statistical analyses^{45,46}, including rigid-body motion correction accounting for signal intensity differences between control and label images⁴⁷, threshold-free outlier exclusion based on head motion⁴⁸ and a single compartment quantification model, assuming that the label decay is determined by the $T1_b$ only³³. For each TP and for the sleep and sleep deprived groups separately, a group-average $T1_b$ was calculated from Hct measurements⁴², which were employed in the rCBF quantification. Each 3D T1-weighted anatomical scan was segmented into gray and white matter tissue probability maps. All CBF maps were rigid-body registered to the gray matter probability maps. A Diffeomorphic Anatomical Registration analysis using Exponentiated Lie algebra (DARTEL)⁴⁹ template was created using the gray and white matter probability maps. The resulting DARTEL transformations were applied to the CBF maps. All transformations were combined into a single 4th degree B-spline interpolation for each scan.

2.7 Voxel-based analysis and additional statistical tests

The rCBF maps were masked slice-wise at 50% of the maximum MNI GM tissue probability, excluding regions prone for susceptibility artifacts: the inferior cerebellum, inferotemporal lobe, and orbitofrontal cortex. Before the voxel-based analysis, the rCBF maps were smoothed using a 6.4x6.4x6.4 mm full-width half-maximum Gaussian kernel, constrained by the mask. General linear models were run to examine the effects of a day of wake and a night of sleep deprivation or sleep on rCBF. The statistical significance was determined using cluster size inference with an initial cluster forming threshold of $p < 0.001$, where clusters with a corrected family-wise error rate of $p < 0.05$ were considered significant. Since the experimental conditions were identical for the sleep group and the sleep group between TP1 and TP2, we first examined the effect of time across all participants for the TP1-TP2 contrast. Next, we conducted group x time interaction analyses for the TP2-TP3 and TP1-TP3 contrasts. These analyses were run to test the hypotheses of the study. We also ran secondary analyses within the sleep group and within the sleep deprivation group separately, to further explore the effects of sleep and sleep deprivation on rCBF.

Averaged rCBF values were computed in clusters showing significant changes across all participants after a day of waking – TP1 versus TP2 – and in clusters showing significant group x time interaction effects for TP2 versus TP3 and TP1 versus TP3. Associations between rCBF within the significant clusters and HOME, ESS, PSQI and KSS scores, intra-individual variation in reaction time and mean reaction time of the attention network task, and times of and intervals between the MRI scans were conducted using SPSS Statistics,

version 25 for Windows (IBM Corp, Armonk, NY, US). Additional linear mixed models for repeated measurements were employed to adjust for potential effects of head motion on rCBF after wake and sleep, summarizing the six parameters of head motion into a single root-mean-square vector.

3. RESULTS

3.1 Characteristics and sleep-wake habits

Table 1 summarizes the participants' characteristics and sleep-related parameters. All participants had a regular sleep-wake cycle and their self-reported sleep durations in the last month and last week were consistent with the average sleep duration of young Norwegian adults⁵⁰. There was no difference between the groups in number of hours of self-reported sleep the night before TP1 or in hours of sleep per night last week and month (all $p>0.44$). Furthermore, the sleep reported by the sleep group did not differ between the night before TP1 and the night before TP3 ($p=0.1$). However, the sleep group reported less sleep the night before TP3 than their average hours of sleep last week and month (both $p<0.001$). Additionally, all participants reported less sleep the night before TP1 than their average hours of sleep in the last week or last month (both $p<0.001$). Whereas the MRI scanning times for TP1 were similar ($p=0.3$) for the sleep group (08h21 ± 00h47) and the sleep deprivation group (08h36 ± 00h36), the deprived group (23h00 ± 00h31) was scanned ~80 minutes later ($p<0.001$) in the evening compared to the sleep group (21h42 ± 00h31), and ~1.5 hours earlier in the next morning (08h13 ± 00h55 and 06h48 ± 00h30 for sleepers and deprived, respectively, $p<0.001$; Figure 1A). Hct differed between the TPs across all participants ($p<0.03$), leading to the blood T1 values described in Table 2, which were used to correct rCBF quantification. Although head motion was small, it was increased for TP3 in the sleep deprived group (average root-mean-square motion 0.097 ± 0.015 mm at TP3 versus 0.070 ± 0.010 and 0.075 ± 0.013 mm for TP1 and TP2, respectively, $p<0.0001$). There were no other head motion differences between groups or TPs (all p

values >0.12).

3.2 Vigilant attention and subjective sleepiness

There were time and time x group interactions effects on the variation in reaction time (time: $F_{2,35} = 15.6$, $p < 0.001$; time x group: $F_{2,35} = 6.9$, $p = 0.003$), mean reaction time (time: $F_{2,35} = 6.9$, $p = 0.003$; time x group: $F_{2,35} = 27.7$, $p < 0.001$), and KSS (time: $F_{2,35} = 28.8$, $p < 0.001$; time x group: $F_{2,35} = 30.8$, $p < 0.001$), with robust increases in the sleep deprivation group at TP3 (Figures 1B-D).

3.3 Resting CBF changes after a day of waking (TP1 versus TP2)

Across all participants ($n=38$), there were increases in rCBF from TP1 to TP2 in four clusters (Figure 2 and Table 3). The rCBF increases within these clusters ranged from 7.5 to 11% and mainly involved bilateral hippocampus and amygdala, bilateral thalamus, and bilateral precentral and postcentral gyrus. There were no areas where rCBF decreased over the course of the day. The rCBF increases within the four clusters remained significant after adjusting for head motion (all $p < 0.002$). There were no significant associations between time of MRI at TP2 and rCBF within the four clusters of Table 3 (all p values > 0.2). Moreover, there were no significant relationships between time from TP1 to TP2 and the rCBF changes within the four clusters from TP1 to TP2 (all p values > 0.7).

3.4 Resting CBF changes from the evening to the next morning (TP2 versus TP3)

The whole-brain analyses for TP2 versus TP3 showed a main effect of time in bilateral

hippocampus and thalamus (Figure 3a and Table 4a). The group x time interaction analyses revealed clusters with significant interaction mainly in bilateral lateral and medial occipital cortices and in bilateral insula (Figure 3b). These effects were driven by rCBF increases in the sleep deprived individuals and decreases in the sleepers, respectively (Table 4b) and remained significant after adjustments for head motion (all $p < 0.001$). There were no significant associations between time of MRI at TP3 and rCBF within the five clusters of Table 4b (all p values > 0.2). Moreover, there were no significant relationships between time from TP2 to TP3 and the rCBF changes within the five clusters from TP2 to TP3 in the sleep group or in the sleep deprived group (all p values > 0.3). We also examined rCBF changes from TP2 to TP3 within the sleep group ($n=19$) and found decreases in bilateral hippocampus and amygdala and right lateral temporal and occipital cortices (Figure 4a). Within the sleep deprived group, there were rCBF increases in bilateral medial occipital and parietal cortices and CBF decreases in bilateral thalamus from TP2 to TP3 (Figure 4b). Figure 5 shows the results of the conjunction analysis of changes from TP1 to TP2 across all individuals and changes from TP2 to TP3 in the sleep group, with overlap for bilateral hippocampus and amygdala.

3.5 Resting CBF changes from the first to the second morning (TP1 versus TP3)

The whole-brain analyses for TP1 versus TP3 showed a main effect of time primarily in right thalamus and bilateral precentral and postcentral gyrus (Figure 6a and Table 5a). The group x time interaction analyses revealed clusters with significant interaction mainly in medial and lateral occipital cortices, in anterior cingulate gyrus and in the insula, in both

hemispheres (Figure 6b). These effects were mainly driven by CBF increases from TP1 to TP3 in the sleep deprived individuals (Table 5b) and remained significant after adjustments for head motion (all p values <0.02). There were no significant associations between time of MRI at TP3 and rCBF within the five clusters of Table 5b (all p values >0.2). Moreover, there were no significant relationships between time from TP1 to TP3 and the rCBF changes within the five clusters from TP1 to TP3 in the sleep group or in the sleep deprived group (all p values >0.2). Within the sleep deprived group, there were CBF increases mainly in medial and lateral occipital cortices, in the insula, and in somatosensory and motor cortices, in both hemispheres (Figure 7). The sleep deprived group also showed CBF decreases from TP1 to TP3, primarily in right thalamus, left cerebellum, and in bilateral lateral parietal cortices. There were no significant CBF changes from TP1 to TP3 in the sleep group (data not shown).

3.6 Resting CBF, sleep-wake characteristics, vigilant attention, and subjective sleepiness after a day of wake and sleep deprivation

We first examined the relationships between rCBF at TP2 within the significant clusters of Table 3 and sleep-wake characteristics, vigilant attention, and subjective sleepiness. Here, there were positive associations between variation in reaction time and rCBF in clusters II ($r=0.32$, $p=0.05$) and IV ($r=0.43$, $p=0.008$) at TP2 across all individuals (Supplemental Table 1). However, none of these remained significant after adjustments for multiple analyses ($N=24$; Bonferroni-corrected significance threshold of $p=0.002$). We then ran analyses for the relationships between changes within the significant clusters of Table 3 from TP1 to

TP2 and changes in variation in reaction time, changes in mean reaction time, and changes in KSS, from TP1 to TP2. Here, there were no significant associations (all $p > 0.24$).

Next, we ran association analyses for the significant clusters of Table 4b (TP2 versus TP3) for the sleep deprived individuals and the sleepers separately. There was a negative association between rCBF of cluster IV in Table 4b and HOME score ($r = -0.65$, $p = 0.003$) in the sleep group (Supplemental Table 2a), yet this was not significant after correcting for multiple analyses (60 analyses (30 per group); Bonferroni-corrected significance threshold of $p = 0.0008$). We then conducted correlation analyses of the relationships between changes within the significant clusters of Table 4b from TP2 to TP3 and changes in variation in reaction time, changes in mean reaction time, and changes in KSS, from TP2 to TP3. These analyses showed a negative association between rCBF changes within cluster IV and changes in mean reaction time in the sleep deprived group ($r = -0.48$, $p = 0.04$); otherwise, there were no significant associations in the sleep group or in the sleep deprived group (all $p > 0.05$).

We also ran association analyses for the significant clusters of Table 5b (TP1 versus TP3). There was a negative association between rCBF of cluster V and HOME score ($r = -0.59$, $p = 0.008$) in the sleepers (Supplemental Table 3a), yet this was not significant after adjusting for multiple analyses (60 analyses; Bonferroni-corrected significance threshold of $p = 0.0008$). Otherwise, all p values were > 0.01 in the sleep group and in the sleep deprivation group (Supplemental Table 3). We then ran correlation analyses of the

relationships between changes within the significant clusters of Table 5b from TP1 to TP3 and changes in variation in reaction time, changes in mean reaction time, and changes in KSS, from TP1 to TP3. Here, there were negative associations between changes in KSS and changes in rCBF of clusters IV ($\rho=-0.50$, $p=0.03$) and V ($\rho=-0.55$, $p=0.01$) in the sleep deprived individuals; otherwise, there were no significant associations in the sleep group or in the sleep deprived group (all $p>0.05$). The latter associations did not remain significant after adjustments for multiple analyses (30 analyses (15 per group); Bonferroni-corrected significance threshold of $p=0.002$).

4. DISCUSSION

In this study, we found that a day of wake – TP1 versus TP2 – was associated with rCBF increases in bilateral hippocampus, amygdala, thalamus, and sensorimotor cortices. The analyses also indicated that these increases were reversed by sleep, particularly those involving the hippocampus and the amygdala. Group (sleep versus sleep deprivation) x time interaction analyses for evening versus next morning (TP2 versus TP3) revealed significant interaction in bilateral lateral and medial occipital cortices and in bilateral insula, driven by further rCBF increases in the sleep deprived individuals and decreases in the sleepers, respectively. Moreover, group x time interaction analyses for first morning versus next morning (TP1 versus TP3) showed significant effects in medial and lateral occipital cortices, in anterior cingulate gyrus, and in the insula, in both hemispheres. These effects were mainly driven by CBF increases from TP1 to TP3 in the sleep deprived individuals. There were no associations between rCBF in significant clusters and sleep-wake characteristics, vigilant attention, and subjective sleepiness that remained significant after adjustments for multiple analyses.

4.1 Resting CBF increases after a day of wake and decreases after a night of sleep

The first main finding of this study was that rCBF increased from morning to evening and decreased after a night of sleep. These rCBF changes were most prominent in the bilateral hippocampus, amygdala, thalamus, and in sensorimotor cortices. There was no difference in rCBF between the first and the second morning sessions of the sleep group, indicating that rCBF resets to the morning level after a night of sleep.

Several studies investigated CBF and CMR during the normal sleep-wake cycle. One Doppler ultrasonography study found decreased CBF after a night of sleep relative to presleep in six participants²⁹. Reduced CBF was also found in 10 participants during sleep using the Kety-Schmidt method⁵¹. Our findings are consistent with a positron emission tomography (PET) study of 20 sleep deprived participants, that found a 18% global rCBF decrease after sleep with most prominent decreases in the occipital and temporal cortices and in the hippocampus³⁰. Another PET study of 22 participants showed a trend towards increased the resting global CMR of glucose (rCMRglc) from morning to afternoon⁵². A mixed pattern of increases and decreases in the metabolism of the cortex and increases in brainstem and hypothalamic arousal centers were found when comparing evening with morning scans in a PET study of 10 participants⁵³. However, a recent PET study of 10 participants quantified rCBF, rCMRglc and resting CMR of O₂ and did not find a difference in flow or metabolic measures between morning and nighttime scans³². Moreover, an ASL study compared rCBF (of the cortical mantle only) in the morning (~9AM) with afternoon (~5PM) scans from another day in 13 participants and found no change in global rCBF³¹, yet reported a regional rCBF decrease from morning to afternoon in a core region of the default mode network (DMN)⁵⁴. Thus, the findings of the present study showing rCBF increases after wake and decreases after sleep are consistent with some^{29,30,51-53}, but not all^{31,32} previous studies. Some of the inconsistencies may result from differences in employed method, study design, and sample size.

Further studies are needed to clarify the precise neural mechanisms underlying sleep-wake

cycle-dependent rCBF changes found in the present study. These rCBF alterations after a day of wake and a night of sleep may be related to sleep-wake-dependent homeostatic factors, circadian variation, or a combination of these processes. Concerning homeostatic factors, a growing body of evidence supports the synaptic homeostasis hypothesis, which posits that wake and sleep are associated with synaptic strengthening and synaptic downscaling, respectively^{17,28}. One important prediction of this hypothesis is that CMR and CBF should increase after wake and decrease after sleep^{17,28}, since synaptic strengthening increases the metabolic cost^{26,27}. Therefore, although speculative, the rCBF changes observed in the present study might, at least in part, be related to diurnal variation in cerebral synaptic strength and metabolic rate.

The rCBF changes in the thalamus after a day of wake and a night of sleep deprivation are of particular interest in relation to SCN-controlled arousal mechanisms of the brain. The dorsal paraventricular thalamus, formerly considered part of “the nonspecific thalamus”, has recently been shown to modulate state-dependent behavior and is a possible anatomical link between hypothalamic control centers for circadian timing and sleep-wake regulation and the cerebral cortex⁵⁵. The thalamic regions that showed rCBF changes after wake in the present study could represent a meeting point for the integration of SCN function onto the cortex. Although the pattern of rCBF changes observed in the present study could be related to sleep-wake-dependent homeostatic factors, e.g., synaptic homeostasis, these findings could also reflect potential SCN-mediated circadian variation in resting brain activity or an integration of these processes.

The findings in the hippocampus and amygdala after wake and sleep, as observed in the present study, are interesting. The hippocampus has well-established roles in learning and memory consolidation^{1,28} and the amygdala is important for emotional learning and memory modulation⁵⁶. Synaptic plasticity mechanisms, such as long-term potentiation, are likely neurobiological substrates for these processes^{1,28,57}. Sleep, on the other hand, facilitates efficient encoding and storage of memories, possibly by synaptic strength regulation, and non-rapid eye movement (REM) sleep is believed to play important roles in renewing the next-day learning capacity^{1,58,59}. Although more research is needed to elucidate the underlying neural processes, the present diurnal rCBF variation findings in the hippocampus and amygdala are in agreement with hypotheses for learning and memory-related synaptic alterations during wake and sleep.

4.2 Resting CBF changes after total sleep deprivation

The second main finding of the present study was that a night of sleep deprivation – TP2 versus TP3 – was associated with further rCBF increases in bilateral lateral and medial occipital cortices and in bilateral insula. In addition, the group x time interaction analyses for first morning versus next morning (TP1 versus TP3) indicated that the sleep deprivation was associated with rCBF increases in medial and lateral occipital cortices, in anterior cingulate gyrus, and in the insula, in both hemispheres.

The first systematic studies of effects of sleep deprivation on human cerebral function were conducted in the late 19th century⁶⁰, yet whether and how lack of sleep affects CBF and CMR

remains to be clarified. An early study measured rCBF in 11 rested participants and 25 individuals sleep deprived for 20 hours with the Kety-Schmidt method and reported a non-significant increase in rCBF in the sleep deprived group⁶¹. A PET study of 17 participants sleep deprived for 24 hours found a decrease in global rCMRglc of 8%, with widespread regional decreases, most prominent in the thalamus and in the frontal and parietal lobes⁶². Two other PET studies detected decreased thalamic metabolism⁶³ and reduced metabolism in the frontal cortex, thalamus, and striatum⁶⁴ after sleep deprivation. An ASL MRI study observed no global effect of partial sleep deprivation, yet reported frontoparietal rCBF decreases in a subgroup of participants with strong signs of drowsiness⁶⁵. Another ASL study of 20 participants found increases in rCBF in early visual cortices after sleep deprivation⁶⁶. Thus, although the number of studies conducted to date is limited, there is some evidence that sleep deprivation leads to rCBF or rCMRglc decreases in frontoparietal cortices as well as in the thalamus, and to rCBF increases in early visual cortices. These observations are – at least partly – consistent with the results of the present study.

The analyses indicated that 23 hours of sleep deprivation was associated with rCBF increases in bilateral insula and anterior cingulate gyrus. These regions are central hubs of the salience network and regulate the anti-correlation between the task-related central executive network and the DMN⁶⁷. It has been hypothesized that sleep deprivation-related changes of insula activity could contribute to the unstable reciprocal inhibition between task-related activity and DMN activity found after insufficient sleep⁶⁸. A recent rodent study also implicated the insula in sleep and wake regulation⁶⁹. Here, lesions of the anterior insula

resulted in decreased wakefulness and increased REM and non-REM sleep. Whether the insula and anterior cingulate gyrus are involved in wake and sleep regulation in humans should be addressed by future studies. We also found rCBF increases in somatosensory and motor cortices after sleep deprivation. These findings are in line with studies showing increased somatosensory evoked potentials^{70,71} and altered motor cortex excitability^{72,73} after sleep deprivation. Although the precise substrates for increased evoked potentials, excitability changes, and rCBF increases within somatosensory and motor cortices remain to be elucidated, these findings together indicate that neural processing within these regions are affected by sleep deprivation.

The analyses within the sleep deprivation group suggested that sleep deprivation is associated with rCBF decreases in parietal association cortices. These findings are consistent with recent functional MRI experiments which have found reduced activity in lateral parietal regions after sleep deprivation during working-memory and attention tasks^{68,74,75}. The analysis restricted to the sleep deprivation group also suggested rCBF decreases in the thalamus, which is consistent with some previous studies detecting reduced thalamic metabolism after sleep deprivation⁶²⁻⁶⁴. The sleep deprivation-related rCBF reduction in the thalamus of the present study may reflect decreased thalamic activity, which could lead to reduced arousal and cortical rCBF reductions .

There were cortical rCBF increases from morning to evening and further increases in bilateral occipital and temporal cortices and insula after a night of sleep deprivation. While

the rCBF increases from morning to evening could reflect sleep-wake-dependent homeostatic factors, circadian variation, or a combination of these processes, it is more likely that the changes in rCBF after sleep deprivation reflect homeostatic mechanisms. Whether these “process S-like” rCBF changes are related to synaptic strength alterations or other neural processes remains to be clarified by future studies.

4.3 Resting CBF, sleep-wake characteristics, vigilant attention, and subjective sleepiness

The analyses for relationships between the rCBF changes and sleep characteristics, vigilant attention, and subjective sleepiness indicated a few noteworthy associations. First, there was a positive association between variation in reaction time and rCBF of clusters II and IV, which included left and right somatosensory and motor cortices, at TP2. Associations between rCBF and drowsiness indices were also observed in a previous sleep deprivation study⁶⁵. However, the latter study found that rCBF decreased in the right fronto-parietal attentional network after partial sleep deprivation in participants with strong signs of drowsiness and that rCBF increased in the basal forebrain and posterior cingulate cortex of participants who remained alert after sleep restriction. The discrepancy between the results of the latter and the present study might be related to experimental design, e.g., partial sleep deprivation⁶⁵ versus total sleep deprivation, and methods, e.g., drowsiness rating based on eye-video recorded during the MRI⁶⁵ versus the attention network task employed in the present study. Second, there was a negative association between HOME score and decreases in rCBF of cluster V, mainly involving the right insula, in the sleep

group, indicating greater rCBF decreases after sleep in individuals with higher “morningness” scores, at TP3. Finally, there was a negative association between changes in KSS score and rCBF changes from TP1 to TP3 in clusters IV and V, primarily involving bilateral insula, indicating smaller KSS score changes in individuals with greater rCBF changes. The latter findings are noteworthy given the potential contributions of the insula to wakefulness regulation. Importantly, however, there were no associations that remained significant after adjustments for multiple analyses and further studies are needed to clarify the relationship between rCBF changes and sleep characteristics, vigilant attention, and subjective sleepiness.

4.4 Methodological considerations and implications for future research

There are several methodological aspects of this study that warrant comment. First, the current study is the largest neuroimaging study to date for rCBF changes after wake, sleep, and sleep deprivation. Second, the participants reported less sleep the night before TP1 than their usual sleep duration. However, assuming that sleep and wakefulness have opposite effects on rCBF, we would expect reduced sleep duration before TP1 to attenuate the waking-related rCBF increases that are observed in this study. Likewise, the fact that the sleep group reported less sleep than usual before TP3 might have resulted in an underestimation of the effects of sleep on rCBF. Third, several factors may affect rCBF measurements⁷⁶, some of which could vary in a rhythmical manner and therefore influence the rCBF measurements in the present study. Although our experiment was already controlled for several potential confounders, including hydration, hct and head motion, a

more rigid control regime could be exerted on potential *Zeitgeber* signals such as physical external stimuli and cognitive workload during wake. Fourth, the sleep group slept at home, whereas the sleep deprivation group was monitored at the hospital. This could have resulted in differences in light exposure and homeostatic cues that, at least in theory, could have influenced the rCBF of the sleep group. Future studies should ideally house all participants in a sleep laboratory under the same environmental conditions. Fifth, there were differences between the sleep and the sleep deprivation groups in the times of the second and third MRI. Although the time differences were relatively small, it remains unclear whether these may have affected the rCBF results. However, there were no significant associations between the rCBF of the significant clusters and the times of and the intervals between the MRI scans. Thus, it seems unlikely that the MRI time differences have significantly influenced the rCBF group differences of this study. Sixth, an important limitation of the present study is that we cannot rule out that participants had brief periods of sleep during the ASL MRI, since no eye tracking or electroencephalography data was collected during scanning. However, we believe it is unlikely that this has substantially affected the results of the present study for several reasons. The ASL sequence was played before structural MRI sequences and we are confident that all participants were awake during the structural scans since they then completed a continuous subtraction task that was monitored by a research assistant. Moreover, the risk of falling asleep should be lower in the early part than in the later part of the MRI session. All participants were asked whether they could have slept during the MRI after completing the scanning sessions, and all reported that they had remained awake. Finally, there is no evidence for sleep-induced

increases in rCBF; thus it seems highly unlikely that rCBF increases after sleep deprivation, as indicated by the present study, are caused by within-scanner sleep. Nonetheless, yet it seems unlikely that the participants had longer periods of sleep during the MRI sessions, participants could have had very brief periods of microsleep that they were not aware took place. Whether the regional rCBF decreases found after sleep deprivation were related to brief microsleep, increased drowsiness, or sleep-wake-dependent homeostatic factors should be clarified by future studies.

Apart from the relevance of our findings within the context of sleep-wake biology, the results of this study indicating morning-evening variation in rCBF may have practical implications for future neuroimaging studies. For example, it is not uncommon that MRI data is acquired from patients in the morning due to the presence of qualified health personnel and from healthy controls in the afternoon and evening. If there indeed exists a systematic variation in rCBF from morning to evening, this could confound group comparisons. Moreover, recent studies also observed time-of-day effects on other MRI modalities^{34-36,77-79}. Future studies should therefore take into account the possibility of time-of-day effects on functional and structural neuroimaging indices.

4.5 Conclusion

The findings of the present study suggest that rCBF increases from morning to evening in bilateral hippocampus, amygdala, thalamus, and sensorimotor cortices and that these changes reverse after a night of sleep. The current study also indicates that sleep

deprivation is associated with further increases in rCBF in occipital and temporal cortices and in the insula. Further studies are needed to clarify the mechanisms underlying rCBF changes after wake, sleep, and sleep deprivation.

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AUTHOR CONTRIBUTIONS

A.B., T.E., and I.R.G., designed the study. T.E., N.Z., L.N., and P.Ø.P. collected the data. T.E., I.R.G., and H.J.M. analyzed the data. T.E., I.R.G. and H.J.M. prepared the manuscript and all authors edited and approved the final manuscript.

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TABLES

Characteristics	All participants (n=38)	Sleep deprived (n=19)	Sleep (n=19)
Age (years)	22.1 ± 2.5	21.5 ± 2.7	22.7 ± 2.2
Handedness (right)	100%	100%	100%
ESS (0-24)	7.3 ± 2.4	7.2 ± 2.6	7.3 ± 2.4
PSQI ^a (0-24)	4.2 ± 2.1	4.0 ± 1.7	4.6 ± 2.5
HOME ^b (16-86)	48.8 ± 8.7	49.0 ± 9.1	48.6 ± 8.4
Daily sleep week before (h:min)	7h30 ± 0h50	7h35 ± 0h50	7h25 ± 1h
Daily sleep month before (h:min)	7h35 ± 0h50	7h35 ± 0h50	7h35 ± 0h50
Sleep before TP1 (h:min)	6h45 ± 1h10	6h55 ± 1h25	6h35 ± 1h
Sleep before TP3 ^b (h:min)	n/a	0	6h10 ± 0h55

Table 1. Sample characteristics and hours of sleep. ESS = Epworth Sleepiness Score, HOME = Horne-Östberg Morningness-Eveningness questionnaire, PSQI = Pittsburgh Sleep Quality Index, n/a = not applicable, TP = time point. ^aMissing for one participant of the sleep group. ^bMissing for one participant of the sleep deprivation group

	Time point 1		Time point 2		Time point 3	
	Hct (%)	T1 _b (ms)	Hct (%)	T1 _b (ms)	Hct (%)	T1 _b (ms)
All participants (n=38)	47.1 ± 2.8	1700 ± 34	45.0 ± 2.5	1729 ± 31	46.1 ± 2.7	1714 ± 33
Sleep (n=19)	46.5 ± 3.0	1709 ± 33	44.7 ± 2.3	1732 ± 30	46.1 ± 2.5	1714 ± 31
Sleep deprivation (n=19)	47.6 ± 2.9	1696 ± 35	45.2 ± 2.7	1726 ± 33	46.2 ± 2.9	1714 ± 36

Table 2. Average hematocrit values (Hct) at each time point and corresponding calculated longitudinal relaxation rate of blood (T1_b), used in the quantification of cerebral blood flow. TP = time point.

Cluster	Cluster volume (mL)	CBF change (%)*	Peak <i>t</i> score	Peak MNI coordinates (mm, for X, Y, Z)	Main brain regions in cluster
I	4.8	11.0	7.6	-19.5, -34.5, 10.5	L hippocampus, L amygdala, B thalamus
II	4.3	9.2	5.5	-46.5, -13.5, 39.0	L precentral gyrus, L postcentral gyrus
III	2.8	7.5	6.1	21.0, -34.5, 9.0	R hippocampus, R thalamus, R amygdala, R lingual gyrus
IV	2.8	8.2	5.1	51.0, -12.0, 33.0	R precentral gyrus, R postcentral gyrus

Table 3. Clusters with significant rCBF increases after a day of wake (TP1 versus TP2; n=38). B = bilateral, L = left, R = right. MNI = Montreal Neurological Institute, rCBF = resting cerebral blood flow, TP = time point. *Positive value indicates increase from TP1 to TP2.

Cluster	Cluster volume (mL)	CBF change (%)*		Peak <i>t</i> score	Peak MNI coordinates (mm, for X, Y, Z)	Main brain regions in cluster
		Sleep deprived group	Sleep group			

A) Cluster with main effect of time from TP2 to TP3:

I	12.1	-9.2	-8.2	6.5	12.0, -27.0, 13.5	B thalamus, B hippocampus
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B) Clusters with significant group x time interaction effects from TP2 to TP3:

I	30.7	9.8	-8.8	6.5	33.0, -79.5, -10.5	R lingual gyrus, R occipital fusiform gyrus, R lateral occipital cortex, R occipital pole, L occipital pole, R temporal occipital fusiform cortex, L cuneal cortex, B lateral occipital cortex, R cuneal cortex, L intracalcarine cortex, R supracalcarine cortex, R inferior temporal gyrus
II	11.8	10.3	-8.7		-33.0, -81.0, -15.0	L occipital fusiform gyrus, L lingual gyrus, L occipital cortex, L occipital pole, L temporal occipital fusiform cortex
III	1.9	7.5	-7.1		-37.5, -40.5, 25.5	L temporal occipital fusiform cortex, L temporal fusiform cortex
IV	1.7	8.2	-8.1	6.2	36.0, 0.0, -10.5	R insular cortex, R central opercular cortex
V	1.5	9.4	-6.6	5.7	-37.5, 4.5, -7.5	L insular cortex, L planum polare

Table 4. A) Cluster with main effect of time from TP2 to TP3 (n=38). **B)** Clusters with significant group x time interaction effects from TP2 to TP3, with CBF increases in sleep deprived individuals and decreases in sleepers (n=38). B = bilateral, L = left, R = right. MNI = Montreal Neurological Institute, rCBF = resting cerebral blood flow, TP = time point. *Positive and negative values indicate increases and decreases from TP2 to TP3, respectively.

Cluster	Cluster volume (mL)	CBF change (%)*		Peak <i>t</i> score	Peak MNI coordinates (mm, for X, Y, Z)	Main brain regions in cluster
		Sleep deprived group	Sleep group			

A) Cluster with main effect of time from TP1 to TP3:

I	4.1	6.3	-3.2	5.18	-40.5, -33.0, 57.0	L postcentral gyrus, L precentral gyrus
II	3.9	7.7	-4.6	5.78	-13.5, -45.0, 69.0	L postcentral gyrus, L superior parietal lobule, L precentral gyrus, L lateral occipital cortex, L juxtapositional lobule cortex, L precuneous cortex
III	1.5	-11.0	-4.1	4.9	10.5, -12.0, 9.0	R thalamus
IV	1.4	6.6	-4.7	5.1	34.5, -27.0, 61.5	R postcentral gyrus, R precentral gyrus

B) Clusters with significant group x time interaction effects from TP1 to TP3:

I	9.4	14.0	-5.4	6.7	51.0, -69.0, -3.0	R lateral occipital cortex, R occipital fusiform gyrus, R lingual gyrus, R temporal occipital fusiform cortex, R occipital pole
II	3.7	14.6	-4.2	6.8	-33.0, -81.0, -16.5	L occipital fusiform gyrus, L lingual gyrus, L lateral occipital cortex
III	3.3	13.3	-3.8	6.0	3.0, 15.0, 28.5	B anterior cingulate gyrus, B juxtapositional lobule
IV	2.2	13.7	-5.3	5.7	-37.5, -4.5, -4.5	L insular cortex, L planum polare
V	1.8	11.1	-5.8	6.6	37.5, -1.5, -10.5	R insular cortex

Table 5. A) Clusters with main effects of time from TP1 to TP3 (n=38). **B)** Clusters with significant group x time interaction effects from TP1 to TP3, with CBF increases in sleep deprived individuals and decreases in sleepers (n=38). B = bilateral, L = left, R = right. MNI = Montreal Neurological Institute, rCBF = resting cerebral blood flow, TP = time point. *Positive and negative values indicate increases and decreases from TP2 to TP3, respectively.

FIGURE LEGENDS

Figure 1. The participants underwent magnetic resonance imaging in the morning after a night of regular sleep in their homes (time point (TP) 1), after approximately 14 hours of waking (TP2), and then 9-10 hours later (TP3), either after a night of sleep at home or after a night of total sleep deprivation (A). Sleep deprivation was associated with significant increases in intra-individual variation in reaction time (B) and mean reaction time (C) of the attention network task and in Karolinska Sleepiness Scale (D). CoV = coefficient of variation, MRI = magnetic resonance imaging.

Figure 2. Resting cerebral blood flow (rCBF) changes after a day of wake (time point (TP1) versus TP2) across all 38 participants, shown in mL/100g/min. Only significant voxels are shown – $p < 0.001$ primary ($k=395$ voxels) and a $p < 0.05$ FWE cluster-forming threshold – overlaid over the population-average anatomical 3D T1 image. There were no areas with significant rCBF decreases. First slice (upper left corner) corresponds to MNI $z=-22$ mm, each increment being 6 mm, ending at MNI $z=68$ mm (lower right corner). The slices are displayed in radiological (left is right) convention. MNI = Montreal Neurological Institute.

Figure 3. Resting cerebral blood flow (rCBF) changes between time point (TP) 2 and TP3. a) shows clusters with main effects of time on rCBF and b) shows clusters with significant group x time interaction, shown in mL/100g/min. Only significant voxels are shown – $p < 0.001$ primary ($k=440$ for both) and $p < 0.05$ FWE secondary cluster-forming threshold – overlaid over the population-average anatomical 3D T1 image. First slice (upper left corner)

corresponds to MNI z=-22 mm, each increment being 6 mm, ending at MNI z=68 mm (lower right corner). The slices are displayed in radiological (left is right) convention. MNI = Montreal Neurological Institute.

Figure 4. Resting cerebral blood flow (rCBF) changes between time point (TP) 2 versus TP3 for a) the sleep group (n=19, left) and b) deprived group (n=19, right), shown in mL/100g/min. Only significant voxels are shown – $p < 0.001$ primary (k=381 and k=390 for a and b, respectively) and $p < 0.05$ FWE secondary cluster-forming threshold – overlaid over the population-average anatomical 3D T1 image. First slice (upper left corner) corresponds to MNI z=-22 mm, each increment being 6 mm, ending at MNI z=68 mm (lower right corner). The slices are displayed in radiological (left is right) convention. MNI = Montreal Neurological Institute.

Figure 5. Conjunction analysis shows the contrasts of Figure 2 (blue), Figure 4a (red) and their overlap (yellow). First slice (upper left corner) corresponds to MNI z=-22 mm, each increment being 6 mm, ending at MNI z=68 mm (lower right corner). The slices are displayed in radiological (left is right) convention. MNI = Montreal Neurological Institute.

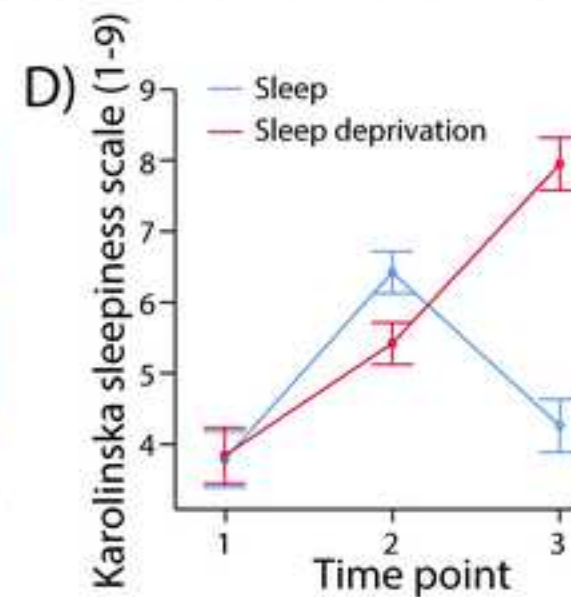
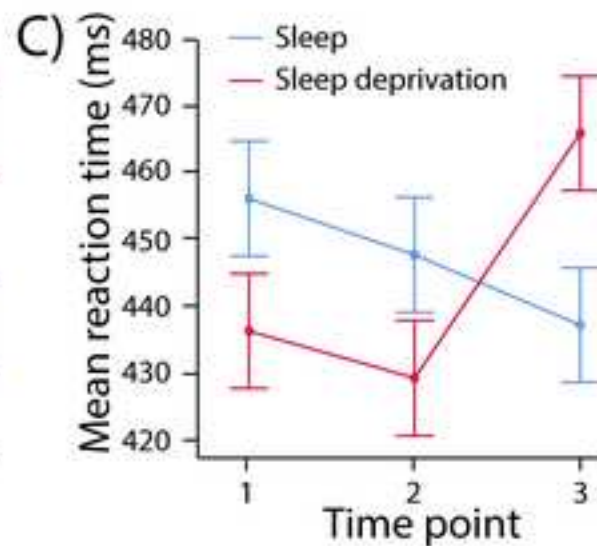
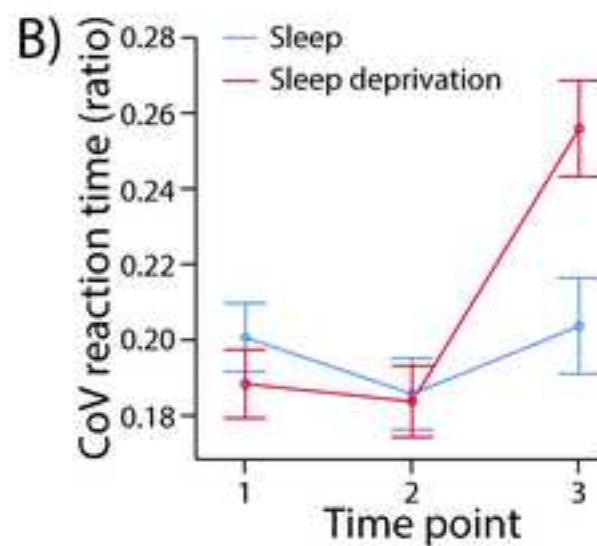
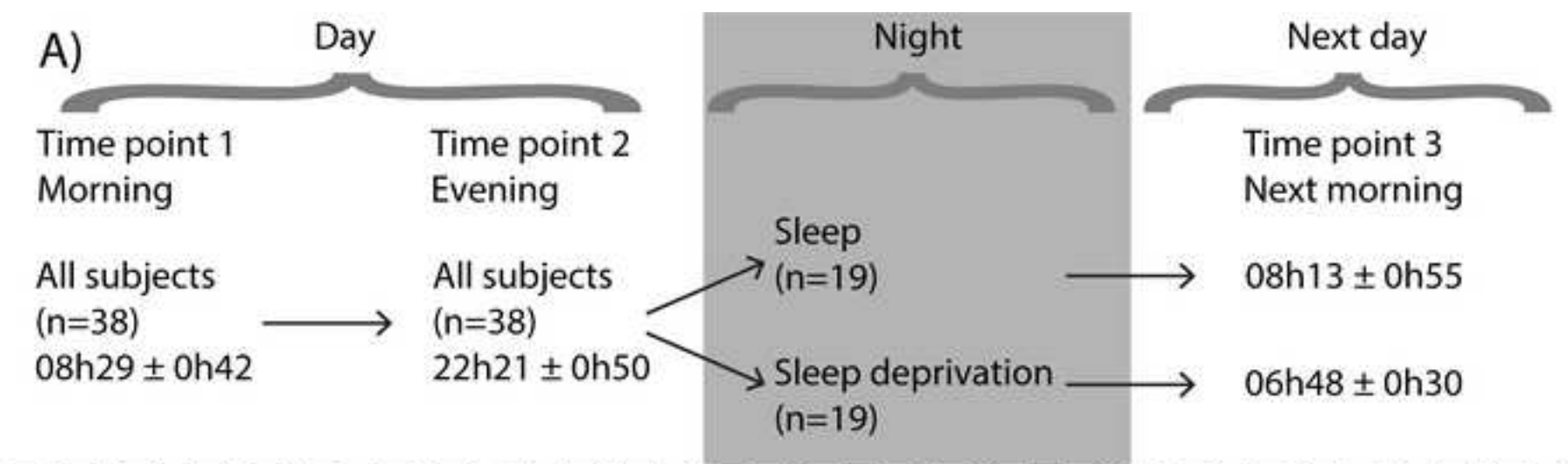
Figure 6. Resting cerebral blood flow (rCBF) changes between time point (TP) 1 and TP3. a) shows clusters with main effects of time on rCBF and b) shows clusters with significant group x time interaction, shown in mL/100g/min. Only significant voxels are shown – $p < 0.001$ primary (k=440 for both) and $p < 0.05$ FWE secondary cluster-forming threshold –

overlaid over the population-average anatomical 3D T1 image. First slice (upper left corner) corresponds to MNI z=-22 mm, each increment being 6 mm, ending at MNI z=68 mm (lower right corner). The slices are displayed in radiological (left is right) convention. MNI = Montreal Neurological Institute.

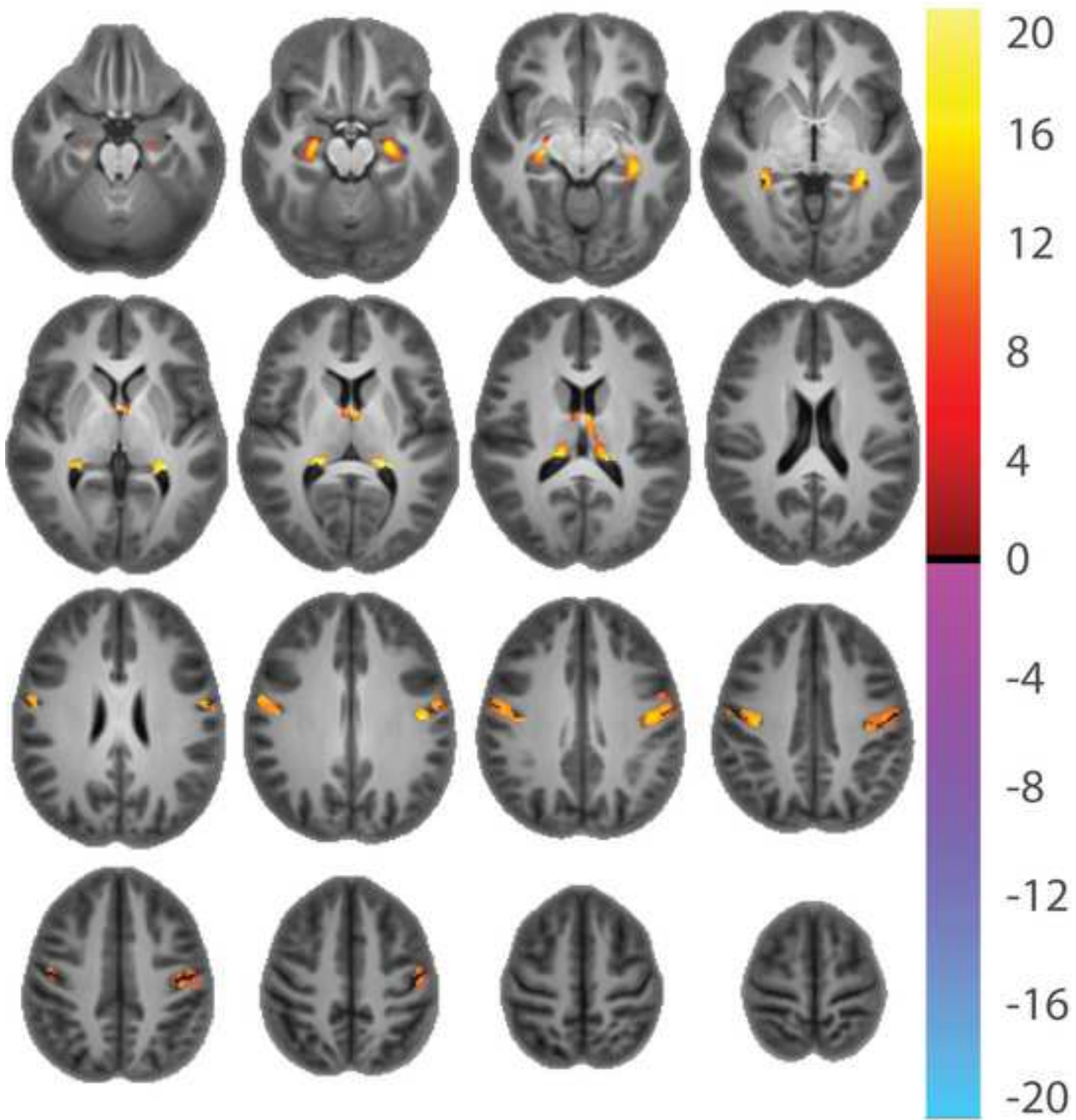
Figure 7. Resting cerebral blood flow (rCBF) changes after approximately 23 hours of sleep deprivation (time point (TP1) versus TP3) in the sleep deprivation group (n=19), shown in mL/100g/min. Only significant voxels are shown – $p < 0.001$ primary (k=283) and $p < 0.05$ FWE secondary cluster-forming threshold – overlaid over the population-average anatomical 3D T1 image. First slice (upper left corner) corresponds to MNI z=-22 mm, each increment being 6 mm, ending at MNI z=68 mm (lower right corner). The slices are displayed in radiological (left is right) convention. MNI = Montreal Neurological Institute. There were no rCBF changes between TP1 and TP3 for the sleep group (n=19, data not shown).

9. Figure 1

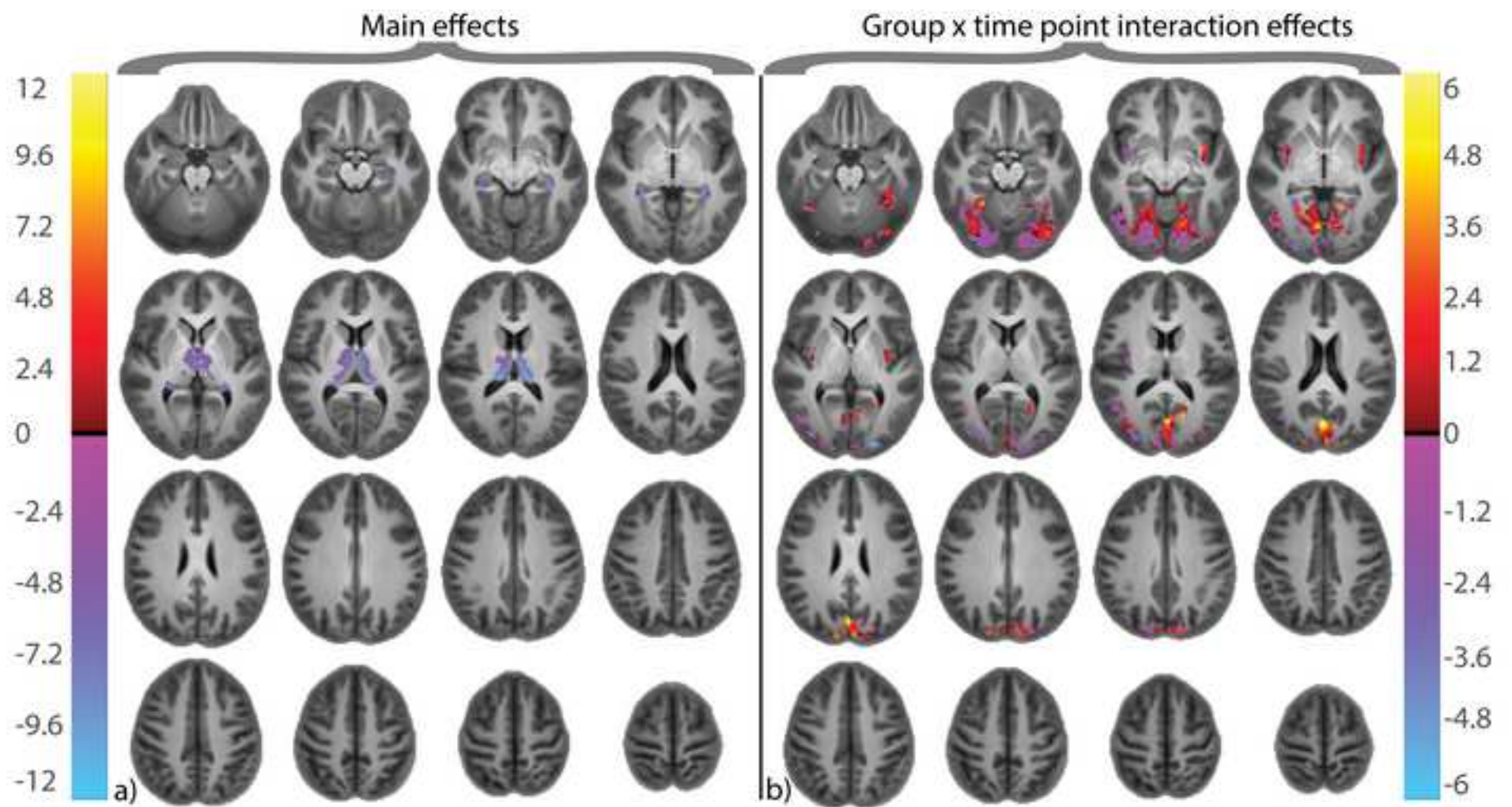
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9. Figure 2
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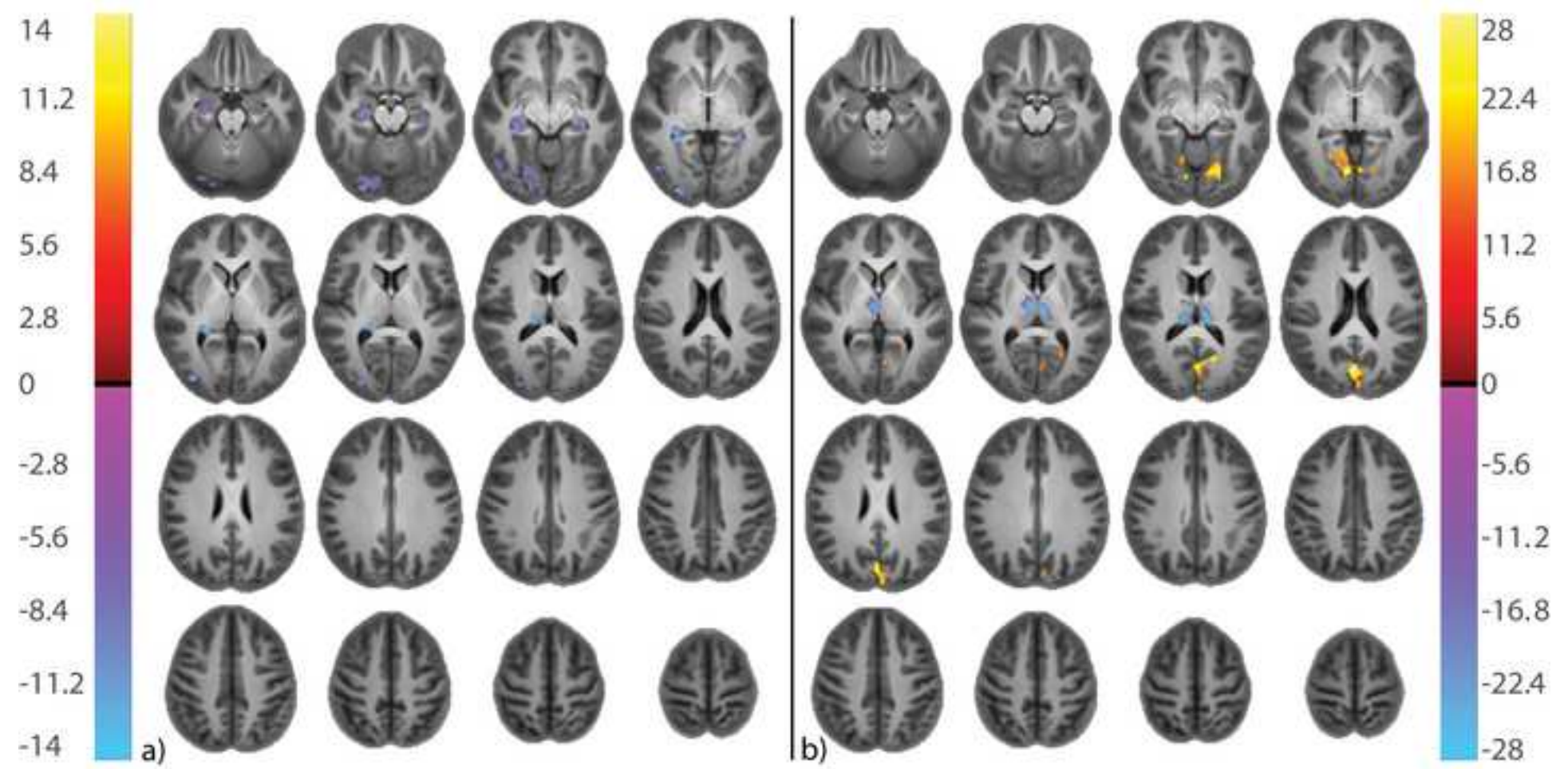


9. Figure 3
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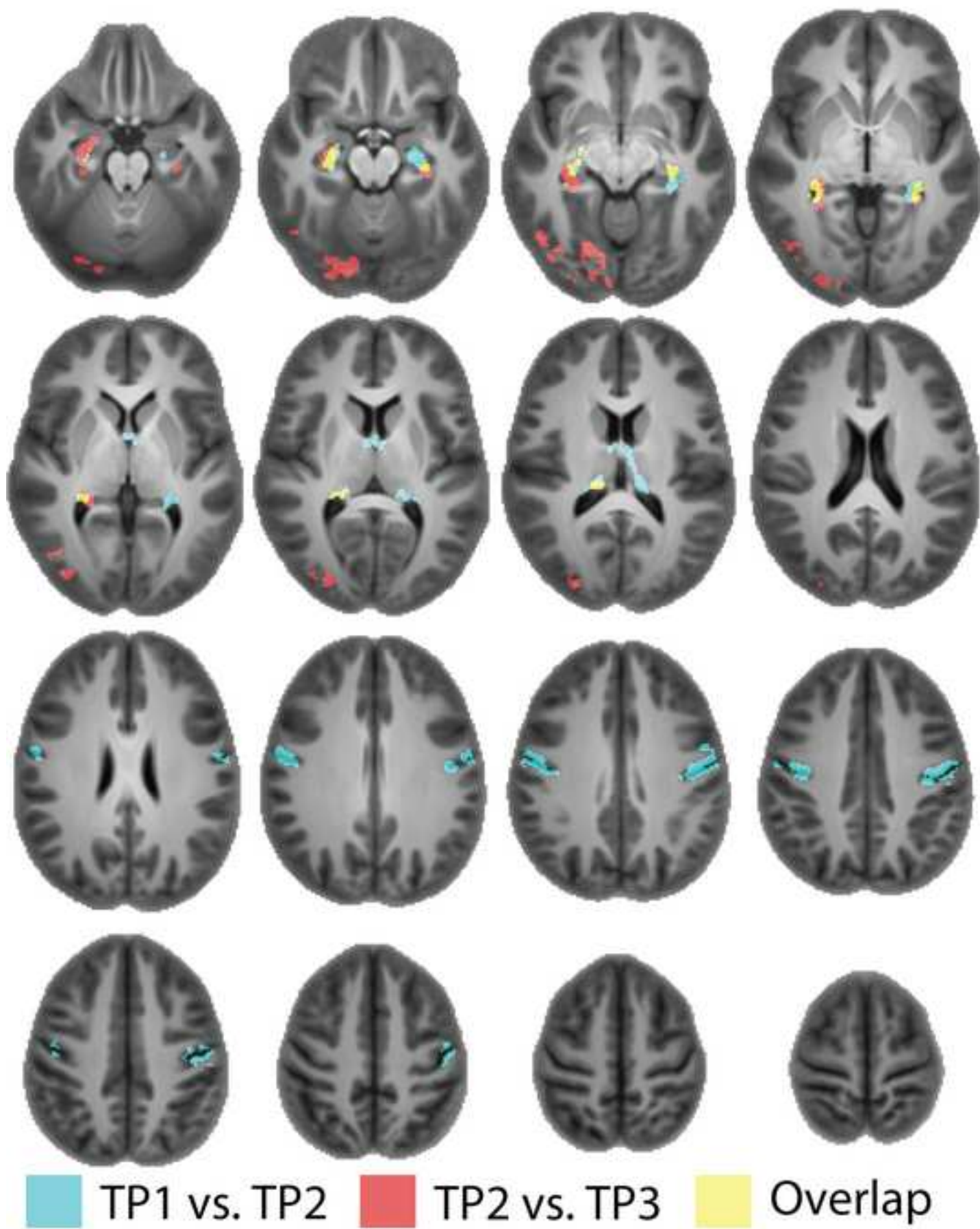


9. Figure 4

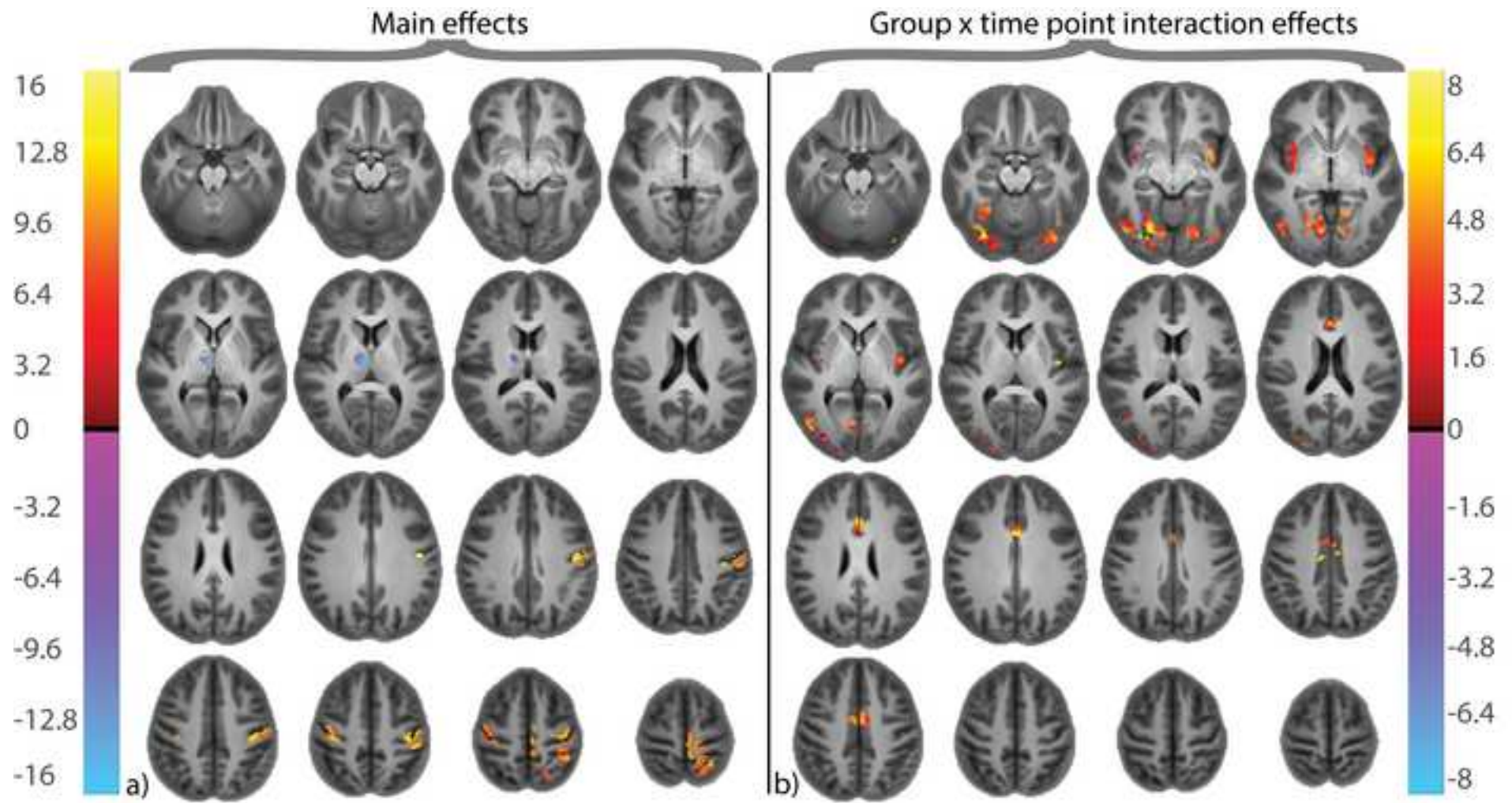
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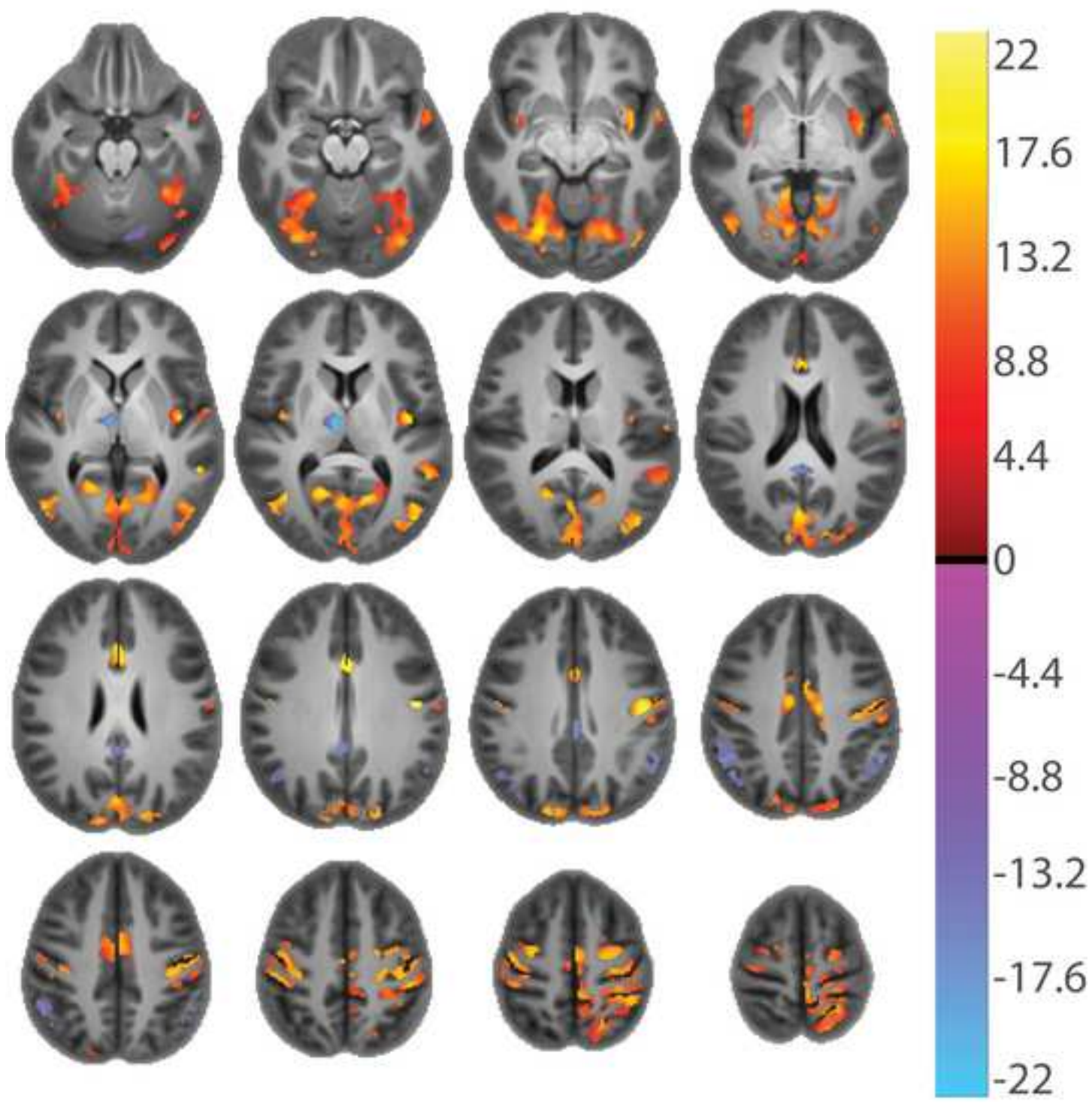
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9. Figure 6
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9. Figure 7
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10. Supplementary Material

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