TITLE

Evidence for cortical structural plasticity in humans after a day of waking and sleep deprivation

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RUNNING TITLE

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ABSTRACT

Sleep is an evolutionarily conserved process required for human health and functioning. Insufficient sleep causes impairments across cognitive domains, and sleep deprivation can have rapid antidepressive effects in mood disorders. However, the neurobiological effects of waking and sleep are not well understood. Recently, animal studies indicated that waking and sleep are associated with substantial cortical structural plasticity. Here, we hypothesized that structural plasticity can be observed after a day of waking and sleep deprivation in the human cerebral cortex. To test this hypothesis, 61 healthy adult males underwent structural magnetic resonance imaging (MRI) 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=41) or a night of sleep (N=20). We found significantly increased right prefrontal cortical thickness from morning to evening across all participants. In addition, pairwise comparisons in the deprived group between the two morning scans showed significant thinning of mainly bilateral medial parietal cortices after 23 hours of sleep deprivation, including the precuneus and posterior cingulate cortex. However, there were no significant group (sleep vs. sleep deprived group) by time interactions and we can therefore not rule out that other mechanisms than sleep deprivation per se underlie the bilateral medial parietal cortical thinning observed in the deprived group. Nonetheless, these cortices are thought to subserve wakefulness, are among the brain regions with highest metabolic rate during wake, and are considered some of the most sensitive cortical regions to a variety of insults. Furthermore, greater thinning within the left medial parietal cluster was associated with increased sleepiness after sleep deprivation. Together, these findings add to a growing body of data showing rapid structural plasticity within the human cerebral cortex detectable with MRI. Further studies are needed to clarify whether cortical thinning is one neural substrate of sleepiness after sleep deprivation.

1. INTRODUCTION

Sleep is an enigmatic, evolutionarily conserved process that constitutes approximately one third of human life.[1-3] Although the precise neural effects of waking and sleep remain to be clarified,[4] robust associations between sleep-wake cycle disruption and altered brain functioning have been documented.[5] Insufficient sleep can cause substantial impairments across cognitive domains in healthy subjects.[6] In addition, sleep-wake cycle disturbances are frequently observed in psychiatric and neurological diseases[7-9] and sleep deprivation can have rapid antidepressive effects in mood disorders.[10,11] Elucidating the neurobiological effects of waking and sleep is therefore an important goal in the basic and clinical neurosciences.

A growing body of evidence from animal studies supports an intimate relationship between structural brain plasticity and the sleep-wake cycle.[12,13] In a landmark study, Xie et al. found that the cortical interstitial space volume was approximately 60% larger in sleeping than in awake mice.[14] Changes in the interstitial space might be consistent with alterations in synaptic volume and number observed in studies of the rodent and fly brain during the sleep-wake cycle.[15-21] In adolescent mice, net increases and decreases in the number of cortical dendritic spines were found after waking and sleep, respectively.[15,16] Studies in flies also provide evidence for changes in synaptic number and volume after hours of waking and sleep.[17-19] Moreover, 16 hours of waking was associated with a 15% increase in the overall volume of the antennal lobes of flies.[18] This finding suggests that macroscopic brain changes can occur within hours of waking, at least in a synapse-rich region of the fly brain.[18] Together, these animal studies indicate that waking and sleep are associated with substantial cortical structural plasticity. However, whether the human cerebral cortex exhibits similar structural plasticity during the sleep-wake cycle remains largely unknown.

There is currently no method available that allows for non-invasive measurement of human cortical interstitial and synaptic volume. An alternative approach for probing structural

cortical plasticity in humans is to use magnetic resonance imaging (MRI)-based morphometry. The unprecedented sensitivity of recently developed MRI-based techniques enables studies of cortical structure with submillimeter accuracy.[22-24] Using these techniques, associations between impaired sleep and cortical grey matter reductions were detected in community-dwelling adults and in sleep disorders.[25-28] In addition, MRI-based evidence for cortical thickening after recovery sleep following a night of sleep deprivation and changes in grey matter volume and thickness from morning to afternoon was recently reported.[29,30] However, whether hours of wake and sleep are associated with alterations in cortical structure detectable with current MRI techniques remains to be fully clarified.

In this study, we hypothesized that structural plasticity can be observed after a day of waking and sleep deprivation in the cerebral cortex of healthy humans. To test this hypothesis, healthy adult males underwent structural MRI at three time points: in the morning after a regular night's sleep, in the evening of the same day, and then the next morning, either after total sleep deprivation (N=41) or a night of sleep (N=20). We found morning-to-evening increase in MRI based thickness of right prefrontal cortices across all participants. In addition, pairwise comparisons in the deprived group between the two morning scans showed significant thinning of mainly bilateral medial parietal cortices after 23 hours of sleep deprivation, including the precuneus and posterior cingulate cortex. Greater thinning within the left medial parietal cluster was associated with increased sleepiness after sleep deprivation. However, we did not detect any significant group (sleep vs. sleep deprived group) by time interaction effects and we can therefore not rule out that other mechanisms than sleep deprivation per se underlie the cortical thinning in the deprived group. Although further studies are needed to confirm cortical thickness changes after a day of waking and to clarify whether cortical thinning is one neural substrate of sleepiness after sleep deprivation, these

findings add to a growing body of data indicating rapid structural plasticity within the human cerebral cortex detectable with MRI.

2. MATERIALS AND METHODS

2.1 Participants

Sixty-one healthy adult males were recruited through local advertising. Exclusion criteria were: history of sleep disorder, neurological or other chronic somatic disorder, current acute somatic illness, psychiatric illness, use of psychotropic drugs, alcohol or drug use disorder, head injury with loss of consciousness for more than one minute, and metallic implants. All subjects had a regular sleep-wake cycle. The participants were assigned either to a sleep deprivation group (N=41) or a sleep group (N=20). There were no significant differences between groups in age or hours of self-reported sleep the last week or the last night before study participation (Table 1). The participants reported less sleep the night prior to study participation and the sleep group reported less sleep before the final MRI session, relative to their average sleep duration the last week (Table 1). Sleep duration estimates were obtained using a self-reporting questionnaire. The reported durations of the participants' sleep are consistent with average sleep length in a recently published self-reporting-based sleep duration study of young Norwegian adults.[31] The Regional Ethical Committee of South-Eastern Norway approved the study, and all subjects provided written informed consent to participate.

2.2 Study protocol

The participants underwent MRI at the Oslo University Hospital 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; Figure 1). The average times of scanning were 8:15AM at TP1, 10:05PM at TP2, and 6:47AM and 8:03AM at TP3 for the non-sleepers and the sleepers, respectively. The sleep deprived group underwent MRI significantly later at TP2 and earlier at TP3, relative to the sleep group (Table 1). No intake of caffeine, nicotine, or alcohol was allowed from the night before the study day until study completion and no intake

of food or energy-containing fluids was allowed the 3 hours before each MRI session. Otherwise, no restrictions were placed on fluid or food intake before or during study participation. Participants were free to leave the hospital after the first MRI session, were instructed not to sleep and to refrain from physical activity, otherwise perform their regular daily activities, and returned at 9PM the same evening for the second MRI session. After the evening examination, the sleep deprivation group stayed overnight at the hospital and was continuously monitored by a research assistant to ensure that none fell asleep. The sleep group left the hospital after the MRI session at TP2, had a night of sleep in their homes and returned to the hospital the next morning for the final MRI session at TP3.

2.3 Assessment of hydration state and sleepiness after sleep deprivation

No gold standard exists for the assessment of hydration state; however, information from two or more hydration indices is recommended for the evaluation of body hydration.[32] In the present study, body weight was measured immediately before imaging and blood samples were drawn immediately after each MRI session for the analysis of plasma osmolality and hematocrit. Sleepiness was assessed at TP3 using the Stanford Sleepiness Scale (SSS), a seven-point rating scale of subjective sleepiness sensitive to sleep deprivation where larger score indicates greater sleepiness.[33] Participants were allowed to report sleepiness using 0.5-scores.

2.4 MRI acquisition

Imaging was performed on a 3T Philips Achieva scanner (Philips Healthcare, Best, the Netherlands). The pulse sequence used for the morphometric analyses was a T1-weighted 3D turbo field echo (TFE) sequence (repetition time/echo time=6.6ms/3.1ms, field of view=256mm×256mm, voxel size=1mm×1mm×1.2mm, flip-angle=8º, acquisition

time=7mins 30s). The sequence was run twice, and the two acquisitions were combined during processing to increase the signal-to-noise ratio (SNR). To reduce head motion, the participants' heads were carefully padded inside the coil. To prevent the subjects from falling asleep during the TFE sequences, the participants completed a subtraction task that required them to press a button at an interval of a few seconds. Each time the button was pressed, a signal was sent to the research assistant, which thereby monitored that the participants were awake. One subject fell asleep before completing the second TFE sequence of the MRI session at TP3. Thus, only the first TFE sequence acquired at TP3 was used in the analysis for this subject. All cortical changes observed in this study remained significant after rerunning the analyses without this subject.

2.5 MRI analysis

Automated cortical surface reconstructions of T1-weighted MR images were performed using FreeSurfer version 5.3 (http://surfer.nmr.mgh.harvard.edu/). Details regarding the surface-based analysis are provided elsewhere.[22-24,34-37] In order to increase sensitivity to within-subject variability, we employed a longitudinal processing scheme.[38] First, datasets from each session were processed independently, including averaging of the two repeated T1 sequences to increase the SNR. Also, several preprocessing steps such as bias field correction, skull stripping, segmentation, and intensity normalization were performed at this stage.[38] Next, an unbiased subject-specific template was generated by combining data from all three time points for each subject, avoiding possible asymmetries or biases.[38] The template was visually inspected for errors and corrected if necessary by a trained research assistant. Subsequently, each time point was aligned with the subject-specific unbiased template and processed[38] resulting in 183 datasets. Thorough inspection and manual edits were performed when necessary for all datasets by the research assistant. This three-steps approach has been shown

to increase sensitivity to longitudinal differences.[38] The individual cortical thickness maps were subsequently resampled onto a common surface (fsaverage), smoothed with a full width at half maximum Gaussian kernel of 15 mm and submitted to higher-level analysis. To obtain an estimate of data quality for each dataset, SNR was computed using Freesurfer QA Tools (http://ftp.nmr.mgh.harvard.edu/fswiki/QATools). Here, SNR is estimated as mean white matter signal divided by the SD of the signal. All MRI preprocessing and manual edits were performed blinded to time of acquisition and group.

2.6 Statistical analyses

Vertex-wise general linear models testing for changes in cortical thickness from morning to evening (TP1 vs. TP2), from the first to the second morning (TP1 vs. TP3), and from the evening to the second morning (TP2 vs. TP3) were performed. The analyses were run separately for each group (sleep and sleep deprived group) and for the combined sample testing for group by time (TP1 vs. TP2, TP1 vs. TP3, and TP2 vs. TP3) interaction effects. In order to reduce the probability for Type I errors, correction for multiple comparisons across the surface was performed, based on cluster size inference by means of Z Monte Carlo simulations.[39] Clusters were tested against an empirical (non-parametric) null distribution of maximum cluster size synthesized across 10000 iterations. The initial cluster-forming threshold employed was $P<0.05$.

Cortical thickness values within significant clusters were exported for additional statistical analyses using SPSS, version 22 for Windows (SPSS, Chicago, Illinois) and a two-tailed $P<0.05$ was considered significant. Then, the relationships between changes in cortical structure from TP1 or TP2 to TP3 and SSS scores at TP3, self-reported sleep duration the night before TP3 or hours between the MRI sessions were examined using Spearman´s rank correlation and Pearson correlation. Finally, linear mixed models were employed to adjust for

potential effects of hydration indices and SNR on changes in cortical thickness after waking and sleep.

3. RESULTS

3.1 Changes in cortical thickness from morning to evening

Fourteen hours of waking (TP1 vs. TP2) was associated with 1.3% mean increase in thickness of a right prefrontal cortical cluster (N=61; 95% confidence interval (CI, 0.6–2.0%); P<0.0001; Figure 2a and Table 2). The vertex-wise analyses did not detect any regions with significant group (sleep vs. deprived group) by time (TP1 vs. TP2) interaction effects (as expected since the study course was the same for the two groups between TP1 and TP2). There were no significant associations between hours from TP1 to TP2 and thickness changes within the right prefrontal cluster in the sleep group, the deprived group, or in the combined sample (all P>0.05).

3.2 Changes in cortical thickness from the first to the second morning

The vertex-wise analyses did not detect any regions with significant group (sleep vs. deprived group) by time (TP1 vs. TP3) interaction effects (Figure 2b).

Within the sleep deprivation group, 1.7% mean decrease (95% CI (0.7–2.6%)) in thickness of a left primarily medial parietal cluster and 1.6% mean thinning (95% CI (0.8–2.5%)) in a right predominantly posteromedial cluster were found after 23 hours of sleep deprivation (TP1 vs. TP3; N=41; both P<0.0001; Figure 2c and Table 2). Cortical thinning in the left and right clusters was strongly correlated (R=0.67, P<0.00001; Figure 3a). There was a significant negative correlation between the thickness change in the left medial parietal cluster and sleepiness at TP3 in the sleep deprivation group (Spearman´s ρ=–0.31, P=0.049; Figure 3b), indicating greater sleepiness in subjects with more pronounced cortical thinning at TP3. Furthermore, there was a trending negative correlation between the thickness change in the right posteromedial cluster and sleepiness at TP3 that was not significant (Spearman´s ρ=–0.23, P=0.149; Figure 3c). There were no significant correlations between hours from

TP1 to TP3 and thickness changes within the left or the right clusters in the sleep group, the deprived group, or in the combined sample (all $P>0.05$).

Within the sleep group, there was 1.4% mean thinning from TP1 to TP3 in a right occipito-temporal cluster ($N=20$; 95% CI (0.6–2.2%); $P=0.0002$; Figure 3d and Table 2). There was no significant association between the thickness change in the occipito-temporal cluster and sleepiness at TP3 (Spearman´s $\rho=0.189$, $P=0.424$). To explore whether sleep duration before the third MRI was related to the occipito-temporal cortical thinning, we correlated self-reported hours of sleep before TP3 with thickness change within the occipito-temporal cluster and found no significant association ($R=–0.137$, $P=0.589$). Moreover, there were no significant correlations between hours from TP1 to TP3 and thickness changes within the occipito-temporal cluster in the sleep group, the deprived group, or in the combined sample (all $P>0.05$).

3.3 Changes in cortical thickness from the evening to the second morning

No significant changes in cortical structure were found in either group from evening to the second morning (TP2 vs. TP3) and there were no significant group (sleep vs. deprived group) by time (TP2 vs. TP3) interaction effects when the analyses were run for the combined sample. However, in the combined sample, there was a trending reversal ($P=0.056$) from TP2 to TP3 of the morning-to-evening thickening (TP1 vs. TP2) within the right prefrontal cluster (shown in Figure 2a). Furthermore, there was a strong negative correlation between thickness changes in the right prefrontal cluster from TP1 to TP2 and the changes within this cluster from TP2 to TP3 among sleepers ($R=–0.63$, $P=0.003$; Figure 3e). A significant, albeit weaker negative correlation between thickness changes in the right prefrontal cluster from TP1 to TP2 and the changes within this cluster from TP2 to TP3 was also found in the sleep deprived group ($R=–0.44$, $P=0.004$; Figure 3f). These correlation coefficients were not significantly different

(Fisher's *Z*-statistics, P=0.36). There were no significant associations between hours from TP2 to TP3 and thickness changes within the right prefrontal cluster in the sleep group, the deprived group, or in the combined sample (all P>0.05).

3.4 SNR and hydration indices

SNR and hydration indices data are summarized in Table 3. There were no significant changes in SNR across the three time points and no significant group by time interaction (all P>0.05). There was higher plasma osmolality (295 vs. 291mOsm/kg), lower hematocrit (44.4 vs. 46.3%), and increased body weight (76.4 vs. 75.9kg) at time point TP 2 relative to TP1 (values averaged across both groups; c. The thickness increase in the right prefrontal cluster from TP1 to TP2 remained significant after adjusting for SNR and the hydration indices (linear mixed models were run both with all hydration indices together and for each index separately; all P<0.05).

There was no difference in plasma osmolality between TP1 and TP3 in the sleep deprivation group (P=0.46). However, hematocrit was lower (45.4 vs. 46.2%) and body weight higher (76.9 vs. 75.9kg) at TP3 than TP1 (both P<0.05). The thickness decrease in the two clusters from TP1 to TP3 remained significant after adjusting for the hydration indices and SNR (all P<0.05).

There was no difference in body weight (P=0.17) or hematocrit (P=0.26) between TP1 and TP3 in the sleep group. Plasma osmolality was significantly higher at TP3 than TP1 in the sleep group (291 vs. 288mOsm/kg). Thickness decrease in the right occipito-temporal cluster from TP1 to TP3 remained significant after adjusting for the hydration indices and SNR (all P<0.05).

4. DISCUSSION

In this study, we tested the hypothesis that a day of waking and sleep deprivation would be associated with alterations in cerebral cortical structure of healthy humans. We found that 14 hours of waking from morning to evening was associated with significant thickening of right prefrontal cortices. We also found thinning of mainly bilateral medial parietal cortices in the sleep deprived group after 23 hours of sleep deprivation. Greater thinning within the left medial parietal cluster was associated with increased sleepiness after sleep deprivation. However, there were no significant group (sleep vs. sleep deprived group) by time interactions and we can therefore not rule out that other mechanisms than sleep deprivation per se underlie the cortical thinning in the deprived group.

4.1 Rapid changes in MRI-derived indices of adult human brain structure

The present findings are consistent with previous studies detecting changes in MRI-derived indices of adult human brain structure within hours to days.[29,30,40-45] Recently, an increase in mean cortical thickness was found after recovery sleep following a night of sleep deprivation.[29] In another recent study, reductions in cortical thickness and grey matter volume from morning to afternoon were detected in healthy volunteers.[30] Furthermore, daily repetitive transcranial magnetic stimulation for 5 days and 7 days of cascade juggling training were associated with localized increases in auditory and occipito-temporal cortical gray matter, respectively.[40,41] A volumetric decrease was found in the ventral putamen of healthy volunteers 1-2 hours after haloperidol infusion; this was partially reversed ~24 hours after drug administration.[42] In another study, 2 hours of spatial learning resulted in decreased mean diffusivity (a diffusion tensor imaging (DTI) measure sensitive to changes in brain microstructure[46]) in the human and rat hippocampus.[43]

The precise anatomical substrate underlying changes in MRI-derived indices of human brain structure observed in the latter studies and the present work has yet to be elucidated. Nonetheless, changes within hours to days might be consistent with the involvement of fast adjusting processes, such as changes in the volumes of glial cells, the cortical interstitial space, and pre- and postsynaptic components of synapses.[14,17,43,47,48] Previous studies found that neuronal activity can induce glial cell changes in both white and grey matter.[49] For example, evidence for astrocytic volume increases was found in the rat optic nerve after electrical stimulation[50] and in the rat hippocampus after spatial learning.[43] Of particular relevance to our findings are previous animal studies indicating changes in cortical interstitial space volume and synaptic structure within hours of waking and sleep.[12-14,20,21] One study found ~60% larger cortical interstitial space volume in sleeping compared to awake mice.[14] Another study reported a 15% overall enlargement of a synapse-rich fly brain region after 16 hours of waking, possibly reflecting a diffuse increase in synaptic volume.[18] The number of cortical synaptic alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, which correlates strongly with the volume of the dendritic spine (the postsynaptic element of the synapse),[51-53] was ~30% higher after hours of waking than after sleep across the rat brain.[54] In several fly brain regions, waking was associated with increases in the volume of presynaptic terminals (the presynaptic element of the synapse) and in dendritic spine number.[17,55] However, whether glial, interstitial space, and synaptic alterations may contribute to changes in MRI-derived structural indices after wake and sleep remains to be clarified and parallel human and animal studies with histological analyses are needed to elucidate the precise underlying mechanisms.

4.2 Structural plasticity in the right prefrontal cortex after 14 hours of waking

We found 1.3% thickness increase in a right prefrontal cluster from morning to evening. This finding may be consistent with neuropsychological and electrophysiological studies indicating that cortical changes during the sleep-wake cycle are particularly prominent in prefrontal regions.[56-61] In addition, changes in cortical thickness from morning to evening are in line with previous works showing time-of-day effects on MRI-derived indices of brain function and structure. Resting-state functional (rs-f) MRI studies observed connectivity strength changes in sensori-motor regions and within subsystems of the default mode network (DMN), including prefrontal and medial parietal regions, from morning to evening in healthy volunteers.[62-64] Another rs-fMRI study found increased connectivity between medial temporal lobes and neocortical regions, including dorsal prefrontal and parietal cortices, in the evening relative to the morning.[65] Widespread morning-to-evening variation was also found in DTI indices of cerebral white matter microstructure in healthy humans.[44,45] In a recent study of longitudinal MRI data from multiple sclerosis trials and the Alzheimer's Disease Neuroimaging Initiative, small, but significant total brain volume decreases after a day of waking were detected (0.09-0.22% decreases per 12 hours).[66] Another recent study obtained MRI-derived indices of brain structure in healthy volunteers from 10AM to 12PM and then again from 2PM to 4 PM.[30] Here, decreases in frontal and temporal cortices thickness and trends towards increases in the thickness of parietal and occipital cortices were found from morning to afternoon.[30] Altogether, while further work is required to clarify the regional characteristics and direction of the effects, these studies indicate that time-of-day can influence MRI-based indices of brain function and structure.

4.3 Bilateral cortical thinning in the sleep deprivation group

The second finding of this study was bilateral cortical thinning after 23 hours of sleep deprivation. However, there were no significant group (sleep vs. sleep deprived group) by

time interactions and we can therefore not rule out other mechanisms than sleep deprivation per se underlying the cortical thinning in the deprived group. Thus, these findings should be considered cautiously and cortical thinning after sleep deprivation need to be confirmed by future research. Notwithstanding this limitation, these results might be consistent with the results of a recent study that observed an increase in mean cortical thickness after recovery sleep following a night of sleep deprivation in healthy individuals.[29] Furthermore, cortical thinning in the sleep deprived group was primarily detected in bilateral posteromedial cortices, including the posterior cingulate cortex (PCC) and the precuneus. These cortices are key nodes in the DMN and are among the most highly connected and metabolically active brain areas.[67-69] It has been suggested that the unusually high metabolism in the PCC and the precuneus is one explanation for their particular vulnerability to pathological conditions such as carbon monoxide poisoning, diffuse brain ischemia, and Alzheimer's disease.[67,70,71] However, whether the high metabolic rate in these cortices could render them especially vulnerable to presumed detrimental effects of sleep deprivation, such as activation of oxidative stress and pro-inflammatory cytokine pathways, and accumulation of neurotoxic molecules, remains to be clarified.[72-75]

Activity of the PCC and the precuneus are believed to support wakefulness and conscious experience.[68,69] The PCC and the precuneus are also among the brain regions with largest deactivations during sleep and anesthesia.[67,76,77] Furthermore, a recent meta-analysis found that the PCC, the precuneus, and the thalamus were the most frequently affected brain regions in disorders of consciousness and that the activity or connectivity of the precuneus may correlate with the severity of consciousness impairment.[78] In line with a possible link between structural integrity of these cortices and wakefulness, we found that increased cortical thinning was associated with greater sleepiness after sleep deprivation. Although interesting, this relationship was weak to moderate, reached statistical significance only in the

left hemisphere, and was based on self-reported sleepiness. Further investigation is therefore needed to clarify whether cortical thinning is one neural substrate of sleepiness after sleep deprivation.

4.4 Cortical thickness changes in the sleep group

The main aim of this study was to examine whether a day of waking and sleep deprivation would result in detectable changes in cortical structure. Therefore, more subjects were included in the sleep deprivation than in the sleep group. The modest number of sleepers might have resulted in insufficient power to detect significant effects of sleep between TP2 and TP3 on cortical structure. Another possible explanation why no significant changes were detected after sleep between TP2 and TP3 could be that the sleep group reported less sleep between TP2 and TP3 than their usual sleep duration (6.1 vs. 7.4 hours). Nonetheless, across sleepers and the non-sleepers, there was a trending reversal from TP2 to TP3 (P=0.056) of the morning-to-evening thickening in the right prefrontal cluster. Furthermore, there was a strong negative correlation between thickness changes within the right prefrontal cluster from TP1 to TP2 and the changes within this cluster from TP2 to TP3 in the sleep group (Figure 3e). Together, these results provide some evidence that sleep is associated with reversal of waking-related cortical thickness changes. However, we also detected a significant, albeit weaker correlation between thickness changes within the right prefrontal cluster from TP1 to TP2 and changes within this cluster from TP2 to TP3 in the deprived group (Figure 3f). Circadian rhythm-related effects can therefore not be ruled out and further studies are needed to separate them, if present, from potential sleep-wake-dependent effects on cortical thickness.

We also found thinning in a right occipito-temporal cluster from TP1 to TP3 in the sleep group. Although speculative, this finding might be related to the fact that the sleepers reported less sleep the night before TP3 than their usual sleep duration. However, an argument

against this hypothesis is the fact that we found no significant association between self-reported hours of sleep before TP3 and changes of the right occipito-temporal cluster within the sleep group. Furthermore, if the human brain is as plastic as previous studies and the present work suggest,[29,30,40-45] then it cannot be ruled out that novel experiences, such as study participation, could lead to detectable day-to-day variations in cortical thickness. Nevertheless, this finding was unexpected and further studies are required to elucidate the effects of insufficient sleep on cortical thickness and whether novel experiences can lead to observable day-to-day changes in cortical structure.

4.5 Limitations

The present findings come with several limitations. First, the number of sleepers was modest and larger studies are needed to clarify the effects of sleep on cortical structure. Second, sleep duration estimates were obtained using a self-reporting questionnaire. Future studies of sleep and partial sleep deprivation effects on cortical structure should determine sleep duration objectively, e.g., by using actigraphy or polysomnography (PSG). Third, the sleepers reported less sleep the night before the last MRI than their usual sleep duration. The modest number of sleepers and their shortened self-reported sleep duration might explain why we found no significant group by time interactions for the left and right hemisphere clusters that were thinner among the non-sleepers after 23 hours of sleep deprivation. Fourth, hydration changes have recently been shown to affect cortical thickness estimates.[79] However, it is unlikely that hydration underlie the waking-related cortical thickness changes observed in this study for several reasons. All waking-related thickness changes remained significant after correcting for the hydration indices. The non-sleepers were, if anything, better hydrated at TP3 than TP1 and showed cortical thinning at TP3. This is the opposite of what would be expected if hydration was the underlying mechanism, since decreased and increased hydration were associated with

widespread, uniform, and symmetric decreases and increases in cortical thickness, respectively.[79] Moreover, it seems unlikely that hydration changes from TP1 to TP2 would result in a localized thickening in right prefrontal cortices and at the same time a trend towards thinning in posteromedial cortices (Figures 2a,c). Fifth, the participants reported less sleep before the first MRI session than their usual sleep duration. However, assuming that sleep and waking have opposite effects on cortical structure, we would expect a reduced sleep duration before the first MRI to attenuate waking-related effects on cortical structure as observed in this study, rather than inflating them. Sixth, we did not examine whether the sleep deprivation-related cortical thinning was reversed by recovery sleep. Finally, future studies should adapt a design with more rigorous control over the subjects' sleep-wake cycle, e.g., by housing subjects in a sleep laboratory, by using PSG recordings, and by including objective assessments of sleepiness and neuropsychological functioning.

4.6 Conclusions

The findings of this study add to a growing body of data indicating substantial structural plasticity in the human cerebral cortex within hours of waking. Further studies are needed to confirm these results and to clarify whether cortical thinning is one neural substrate of sleepiness after sleep deprivation.

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AUTHOR CONTRIBUTIONS

T.E., N.Z., A.B., U.F.M, I.R.G., and L.T.W. designed the study. T.E., N.Z., L.B.N., P.Ø.P., and SHQ collected the data. T.E., L.B.N., D.A., N.T.D., and L.T.W. analyzed the data. T.E. prepared the manuscript and all authors edited and approved the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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FIGURE LEGENDS

Figure 1. Study protocol. 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).

Figure 2. Changes in cortical thickness after 14 hours of waking from morning to evening and 23 hours of sleep deprivation. (**a**) Fourteen hours of waking from time point (TP) 1 to TP2 was associated with 1.3% increase in thickness of a right prefrontal cortical cluster mainly comprising right superior frontal gyrus, rostral middle frontal gyrus, and pars triangularis of the inferior frontal gyrus. The bar chart to the right shows thickness within this cluster at TP2 and TP3 relative to TP1 for the sleep and the sleep deprivation group and for the two groups combined. Across the two groups, there was a trend towards a reversal from TP2 to TP3 of the morning-to-evening thickening (TP1 to TP2) in the right prefrontal cluster. (**b**) There were no regions with significant group (sleep vs. deprived group) by time (TP1 vs. TP3) interaction effects when cortical thickness at TP1 and TP3 was compared. Thus, we cannot rule out other mechanisms than sleep deprivation per se underlying the cortical thinning in the deprived group, as shown in (c). (**c**) Twenty-three hours of sleep deprivation was associated with 1.7% mean decrease in thickness of a left primarily medial parietal cluster including the precuneus, the posterior cingulate cortex, the paracentral lobule, and the superior parietal lobule and with 1.6% mean thinning in a right predominantly posteromedial cluster comprising the precuneus, the posterior cingulate cortex, the paracentral lobule, the superior parietal lobule, the cuneus, and the pericalcarine cortex. The bar charts in the lower part of the figure show thickness within these clusters at TP2 and TP3 relative to TP1.

*P<0.0001.

Figure 3. Cortical thickness changes and sleepiness after sleep deprivation, changes in cortical thickness in the sleep group, and association between morning-to-evening and evening-to-morning changes within the right prefrontal cluster. (**a**) Cortical thinning in the left and right hemisphere clusters from time point (TP) 1 to TP3 in the sleep deprivation group was strongly correlated. (**b**) There was a significant negative correlation between the thickness change in the left medial parietal cluster and sleepiness at TP3 in the sleep deprivation group, indicating greater sleepiness in subjects with more pronounced cortical thinning after sleep deprivation. (**c**) There was a trending negative correlation between the thickness change in the right posteromedial cluster and sleepiness at TP3 in the sleep deprivation group. (**d**) There was significant thinning from TP1 to TP3 in a right occipito-temporal cluster in the sleep group. (**e**) There was a strong negative correlation between thickness changes in the right prefrontal cluster from TP1 to TP2 (Figure 2a) and the changes within this cluster from TP2 to TP3 in the sleep group. (**f**) A significant, yet weaker negative correlation between thickness changes in the right prefrontal cluster from TP1 to TP2 and the changes within this cluster from TP2 to TP3 was also found in the sleep deprived group.

TABLES

Table 1. Characteristics of the participants.

Characteristics	Sleep deprivation group	Sleep group	*P*-value[a]
N	41	20	NA
% Male	100	100	NA
Mean age (years; s.d.)	21.8 (2.4)	22.7 (2.1)	0.17
Average hours of sleep per night last week (s.d.)	7.5 (1.0)	7.4 (0.8)	0.74
Hours of sleep the night before TP1 (s.d.)*	6.6 (1.3)	6.6 (1.0)	0.83
Hours of sleep the night before TP3 (s.d.)[b,]**	NA	6.1 (1.2)	
Time[c] of first MRI (s.d.)	8.17AM (34mins)	8.13AM (44mins)	0.72
Time[c] of second MRI (s.d.)	10.20PM (41mins)	9.33PM (29mins)	<0.001
Time[c] of third MRI (s.d.)[d]	6.47AM (27mins)	8.03AM (56mins)	<0.001

Abbreviations: TP, Time point. MRI, Magnetic resonance imaging. [a]Sleep deprivation group vs. sleep group. [b]Data missing for two participants. [c]The time when MRI was started. [d]Data missing for one participant.

*Across groups, there was less sleep the night before than the week before study participation (*P*<0.05*)*. **Participants in the sleep group slept less the night before TP3 than the week before study participation (*P*<0.001*)*.

Table 2. Clusters with significant changes in thickness after waking and sleep.

Cluster	No. of voxels in cluster	Cluster *P*-value	Regions within cluster	Brodmann's areas within cluster
Right prefrontal cluster with thickening from TP1 to TP2 across all participants	8280	<0.0001	Right superior frontal gyrus, rostral middle frontal gyrus, pars triangularis of the inferior frontal gyrus	9, 10, 45, 46
Left medial parietal cluster with thinning from TP1 to TP3 in the sleep deprivation group	12341	<0.0001	Precuneus, posterior cingulate cortex, paracentral lobule, superior parietal lobule	5,7, 23, 31
Right posteromedial cluster with thinning from TP1 to TP3 in the sleep deprivation group	10003	<0.0001	Precuneus, posterior cingulate cortex, paracentral lobule, superior parietal lobule, cuneus, pericalcarine cortex	5,7, 17, 18, 23, 31
Right occipitotemporal cluster with thinning from TP1 to TP3 in the sleep group	4094	0.0002	Cuneus, pericalcarine cortex, lingual gyrus	17, 18, 19

Abbreviations: TP, Time point.

Table 3. Signal-to-noise ratio of the magnetic resonance imaging data and hydration indices.

Characteristics	Sleep deprivation group			Sleep group		
	TP1	**TP2***	**TP3****	**TP1**	**TP2***	**TP3*****
SNR of the MRI data (s.d.)	21.1 (2.3)	21.3 (2.3)	21.0 (2.2)	21.6 (2.0)	21.3 (1.9)	21.2 (1.9)
Body weight (kg; s.d.)[a]	75.9 (9.8)	76.4 (9.6)	76.9 (9.8)	75.5 (10.4)	76.4 (10.8)	75.7 (10.5)
Hematocrit (%; s.d.)[b]	46.2 (0.03)	44.3 (0.02)	45.4 (0.03)	46.6 (0.03)	44.6 (0.02)	46.0 (0.03)
Plasma osmolality (mOsm/kg; s.d.)[c]	292.7 (5.8)	295.5 (5.1)	292.0 (5.6)	288.1 (3.4)	292.8 (4.3)	291.2 (2.8)

Abbreviations: TP, Time point; SNR, signal-to-noise ratio. [a]Data missing for one sleeper at TP2. [b]Data missing for one non-sleeper at TP1-3, for one non-sleeper at TP1, and for one sleeper at TP3. [c]Data missing for one sleeper at TP3.

*Across groups, plasma osmolality and body weight was higher and hematocrit lower at TP2 than TP1 (both $P<0.05$).

**Hematocrit was lower and body weight higher in the sleep deprivation group at TP3 than TP1 (both $P<0.05$).

***Plasma osmolality was higher in the sleep group at TP3 than TP1 ($P<0.05$).

Figure 1

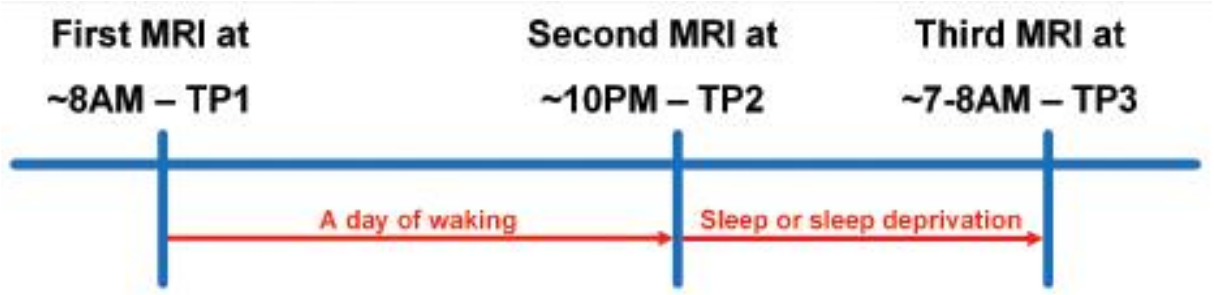

First MRI at
~8AM – TP1
Second MRI at
~10PM – TP2
Third MRI at
~7-8AM – TP3
A day of waking
Sleep or sleep deprivation

Figure 2

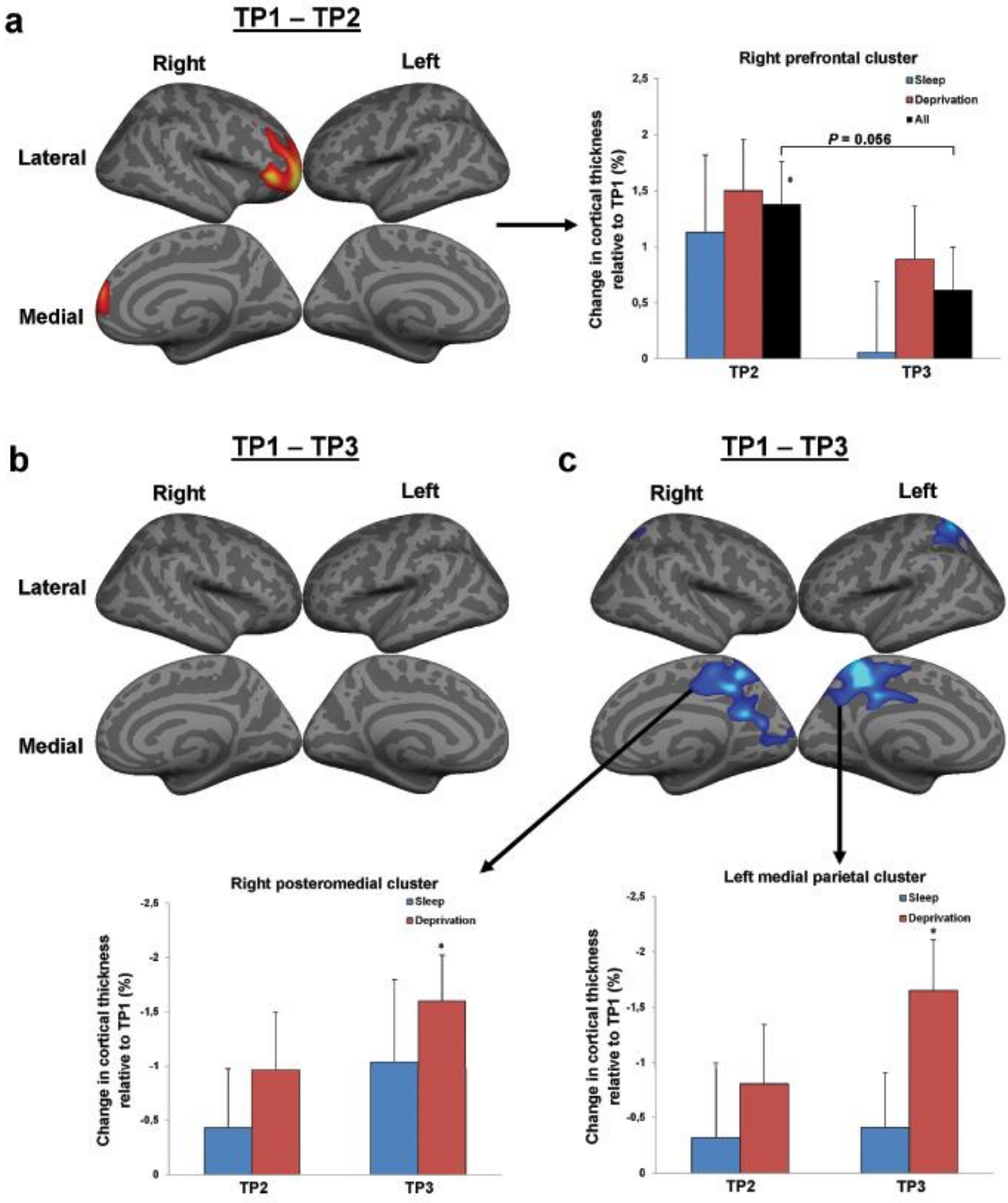
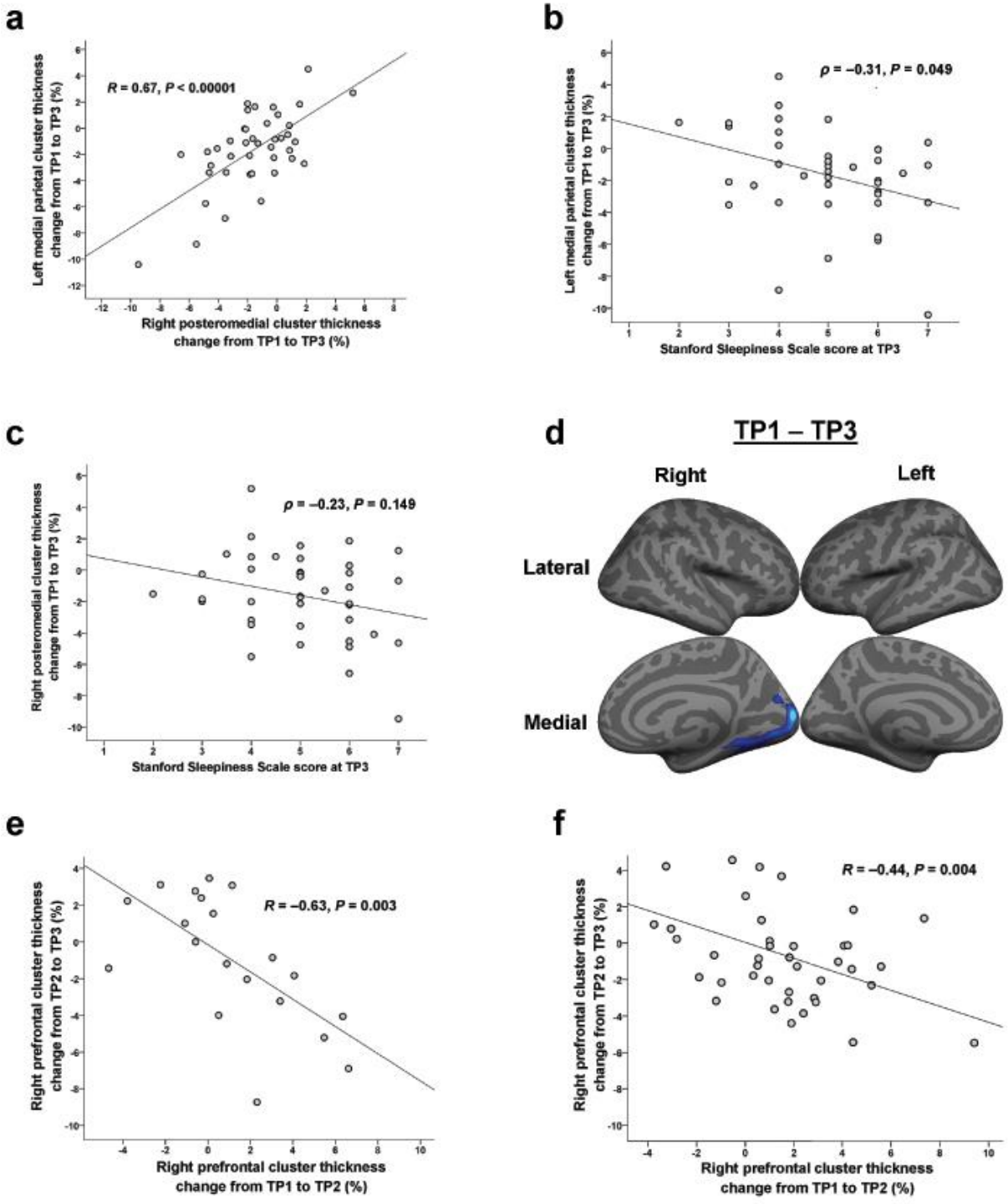

a
TP1 – TP2
Right
Left
Lateral
Medial
Right prefrontal cluster
Sleep
Deprivation
All
P = 0.056
Change in cortical thickness relative to TP1 (%)
2,5
2
1,5
1
0,5
0
TP2
TP3
b
TP1 – TP3
Right
Left
Lateral
Medial
Right posteromedial cluster
Sleep
Deprivation
Change in cortical thickness relative to TP1 (%)
-2,5
-2
-1,5
-1
-0,5
0
TP2
TP3
c
TP1 – TP3
Right
Left
Left medial parietal cluster
Sleep
Deprivation
Change in cortical thickness relative to TP1 (%)
-2,5
-2
-1,5
-1
-0,5
0
TP2
TP3

Figure 3


a
R = 0.67, P < 0.00001
Left medial parietal cluster thickness change from TP1 to TP3 (%)
Right posteromedial cluster thickness change from TP1 to TP3 (%)
b
ρ = –0.31, P = 0.049
Left medial parietal cluster thickness change from TP1 to TP3 (%)
Stanford Sleepiness Scale score at TP3
c
ρ = –0.23, P = 0.149
Right posteromedial cluster thickness change from TP1 to TP3 (%)
Stanford Sleepiness Scale score at TP3
d
TP1 – TP3
Right
Left
Lateral
Medial
e
R = –0.63, P = 0.003
Right prefrontal cluster thickness change from TP2 to TP3 (%)
Right prefrontal cluster thickness change from TP1 to TP2 (%)
f
R = –0.44, P = 0.004
Right prefrontal cluster thickness change from TP2 to TP3 (%)
Right prefrontal cluster thickness change from TP1 to TP2 (%)