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Sleep deprivation impairs molecular clearance from the human brain

Per Kristian Eide,[1,2] Vegard Vinje,[3] Are Hugo Pripp,[4] Kent-Andre Mardal[3,5] and Geir Ringstad[6]

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It remains an enigma why human beings spend one-third of their life asleep. Experimental data suggest that sleep is required for clearance of waste products from brain metabolism. This has, however, never been verified in humans. The primary aim of the present study was to examine *in vivo* whether one night of total sleep deprivation affects molecular clearance from the human brain. Secondarily, we examined whether clearance was affected by subsequent sleep. Multiphase MRI with standardized T_1 sequences was performed up to 48 h after intrathecal administration of the contrast agent gadobutrol (0.5 ml of 1 mmol/ml), which served as a tracer molecule. Using FreeSurfer software, we quantified tracer enrichment within 85 brain regions as percentage change from baseline of normalized T_1 signals. The cerebral tracer enrichment was compared between two cohorts of individuals; one cohort ($n = 7$) underwent total sleep deprivation from Day 1 to Day 2 (sleep deprivation group) while an age and gender-matched control group ($n = 17$; sleep group) was allowed free sleep from Day 1 to Day 2. From Day 2 to 3 all individuals were allowed free sleep. The tracer enriched the brains of the two groups similarly. Sleep deprivation was the sole intervention. One night of sleep deprivation impaired clearance of the tracer substance from most brain regions, including the cerebral cortex, white matter and limbic structures, as demonstrated on the morning of Day 2 after intervention (sleep deprivation/sleep). Moreover, the impaired cerebral clearance in the sleep deprivation group was not compensated by subsequent sleep from Day 2 to 3. The present results provide *in vivo* evidence that one night of total sleep deprivation impairs molecular clearance from the human brain, and that humans do not catch up on lost sleep.

1 Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway
2 Department of Neurosurgery, Oslo University Hospital – Rikshospitalet, Oslo, Norway
3 Center for Biomedical Computing, Simula Research Laboratory, Lysaker, Norway
4 Oslo Centre of Biostatistics and Epidemiology, Research Support Services, Oslo University Hospital, Oslo, Norway
5 Department of Mathematics, University of Oslo, Oslo, Norway
6 Department of Radiology, Oslo University Hospital – Rikshospitalet, Oslo, Norway

Correspondence to: Professor Per Kristian Eide, MD PhD
Department of Neurosurgery
Oslo University Hospital – Rikshospitalet
Pb 4950 Nydalen
N-0424 Oslo, Norway
E-mail: p.k.eide@medisin.uio.no

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Introduction

Sleep is essential for human life, including cognitive function (Rasch and Born, 2013), but it remains a mystery why man spends about one-third of life asleep. Experimental evidence has shown that sleep has a restorative function by facilitating clearance of metabolic waste products from the brain that accumulate during wakefulness (Xie *et al.*, 2013). With two-photon microscopy, it was found that sleep increased the brain interstitial volume fraction by 60%, allowing for a 2-fold faster clearance of amyloid-β from the cortex. The observations echoed with reports of disrupted sleep in the preclinical stage of Alzheimer's disease (Moran *et al.*, 2005), a disease in which amyloid-β and tau aggregation in susceptible brain areas develop long before onset of clinical dementia. Severe sleep disturbances also accompany traumatic brain injury (Mathias and Alvaro, 2012), where patients suffer from increased cerebral tau and amyloid-β burden and risk of Alzheimer's disease (Johnson *et al.*, 2012). It was previously demonstrated in a mouse Alzheimer's disease model that interstitial levels of amyloid-β, a metabolic by-product of neuronal activity, increased after acute sleep deprivation, while chronic sleep deprivation increased amyloid-β formation (Kang *et al.*, 2009). Others also reported that sleep deprivation increased the amount of soluble amyloid-β and the risk of amyloid-β plaque formation in mice (Roh *et al.*, 2012). The levels of tau were also increased in the interstitial fluid of the hippocampus following sleep deprivation (Holth *et al.*, 2019). In healthy humans, undisturbed sleep caused 6% reduction in CSF amyloid-β_{42} levels while remaining unchanged after one night of total sleep deprivation (Ooms *et al.*, 2014). An amyloid-β PET study showed that one night of sleep deprivation increased parenchymal amyloid-β burden by 5% in 20 healthy individuals (Shokri-Kojori *et al.*, 2018). More recently, a direct link between sleep-related neuronal activity and CSF and blood flow was indicated based on observations that slow-wave sleep was accompanied with large-amplitude CSF flow as compared with the awake state, and an inverse relationship between CSF flow and blood flow (Fultz *et al.*, 2019). It has, however, never been demonstrated *in vivo* whether sleep, or sleep deprivation, affects molecular clearance from the human brain.

The present study was undertaken to examine the effect of one night of total sleep deprivation on molecular clearance from the human brain. The MRI contrast agent gadobutrol (Gadovist®, Bayer) was used as tracer molecule to enrich brain tissue via intrathecal administration in CSF (Ringstad *et al.*, 2018). Gadobutrol is a highly hydrophilic molecule with molecular weight of 604 Da, hydraulic diameter of ∼2 nm; and distributes freely within the brain, confined to the extravascular compartment by the blood–brain barrier (Ringstad *et al.*, 2018). Gadobutrol as a CSF tracer may therefore be considered a surrogate marker for assessing transport of water-soluble metabolites excreted along extravascular pathways within the brain, including amyloid-β and tau. The MRI research protocol included standardized T_1-weighted MRI scanning before and after intrathecal gadobutrol at predefined time points during Day 1 and at 24 h (next morning), 48 h (the morning after) and at 4 weeks (Supplementary Fig. 1). Following completion of imaging, the entire brain was analysed in FreeSurfer, allowing for the assessment of 85 subregions.

Materials and methods

The study was approved by the Regional Committee for Medical and Health Research Ethics (REK) of Health Region South-East, Norway (2015/96), the Institutional Review Board of Oslo University Hospital (2015/1868), the National Medicines Agency (15/04932-7), and was registered in Oslo University Hospital Research Registry (ePhorte 2015/1868). The conduct of the study was governed by ethical standards according to the Declaration of Helsinki of 1975 (and as revised in 1983). Study participants were included after written and oral informed consent.

Experimental design

The aim of the study was to determine whether sleep deprivation results in impaired molecular clearance from the human brain. Total sleep deprivation for 24 h in one of the groups (sleep deprivation group) was the intervention. The participants undergoing sleep deprivation were observed within the department of neurosurgery by the nursing staff. Also, a close relative stayed with the participant throughout the night to help them stay awake. Thereby, it was controlled that the participants were awake from Day 1 to Day 2.

An MRI contrast agent, gadobutrol, was administered intrathecally, and served as CSF tracer. MRI acquisitions were carried out at multiple time points: pre-contrast, and after 0–1.5 h, 1.5–3 h, 4.5–7 h (Day 1), 24 h (Day 2), 48 h (Day 3), and after 4 weeks. Individuals allocated to total sleep deprivation were awake from Day 1 until Day 2. From Day 2 until Day 3, they were allowed to sleep without restrictions. The sleep deprivation group was compared with subjects with no restrictions on sleep (sleep group), who were asleep from the evening of Day 1 until the morning of Day 2, during which time the subjective sleep quality was recorded. An illustration of the study design is presented in Supplementary Fig. 1.

Patients

Intrathecal administration of gadobutrol is currently performed off-label on clinical indication and is not used in healthy individuals. Therefore, the study was restricted to include patients under clinical work-up of tentative CSF disorders in the Department of Neurosurgery, Oslo University Hospital – Rikshospitalet (Table 1) and with a clinical indication for performing intrathecal contrast-enhanced MRI. We invited consecutive patients to undergo sleep deprivation; participants in the sleep group included patients who matched the sleep deprivation group regarding tentative diagnosis, age and gender. Other selection criteria were not used. Exclusion criteria included: history of hypersensitivity reactions to contrast agents, history of severe allergy reactions in general, evidence of renal

Table 1 Demographic and clinical information about the two study groups

	Sleep group	Sleep deprivation group	Significance
n	17	7	
Sex, female/male	15/2	6/1	ns
Age, years	39.2 ± 14.1	44.7 ± 15.7	ns
BMI, kg/m^2	28.3 ± 6.2	26.2 ± 3.7	ns
Tentative diagnosis			
Non-verified CSF leakage	3 (17.6%)	2 (28.6%)	ns
Pineal cyst (non-surgery)	5 (29.4%)	1 (14.3%)	ns
Arachnoid cyst (non-surgery)	1 (5.9%)	1 (14.3%)	ns
Hydrocephalus (non-surgery)	1 (5.9%)	0	ns
IIH (non-surgery)	2 (11.8%)	1 (14.3%)	ns
IIH (surgery)	5 (29.4%)	2 (28.6%)	ns
Sleep Day 1 to 2			
Total sleep deprivation	–	7	–
Hours with sleep	6.4 ± 1.9	0	–
Subjective sleep quality Day 1 to 2			
Light	2	–	–
Medium	5	–	–
Deep	10	–	–

Categorical data presented as numbers; continuous data presented as mean ± standard deviation. Significant differences between groups were determined by independent samples *t*-test for continuous data and by Pearson χ^2 test for categorical data. BMI = body mass index; IIH = idiopathic intracranial hypertension; ns = non-significant. The subjective sleep quality from Day 1 to Day 2 is indicated.

dysfunction, pregnant or breastfeeding females, and age <18 or >80 years.

MRI protocol

The study used a 3 T Philips Ingenia MRI scanner (Philips Medical systems), applying equal imaging protocol settings at all time points to acquire sagittal 3D T_1-weighted volume scans. The following imaging parameters were used: repetition time = 'shortest' (typically 5.1 ms), echo time = 'shortest' (typically 2.3 ms), flip angle = 8°, field of view = 256 × 256 cm and matrix = 256 × 256 pixels (reconstructed 512 × 512). We sampled 184 overcontiguous (overlapping) slices with 1 mm thickness that were automatically reconstructed to 368 slices with 0.5 mm thickness; total duration of each image acquisition was 6 min and 29 s. To secure consistency and reproducibility of the MRI slice placement and orientation, slice orientation of image stacks was defined using an automated anatomy recognition protocol based on landmark detection in MRI data (SmartExam™, Philips Medical Systems) for every time point.

Intrathecal gadobutrol as CSF tracer

After the pre-contrast MRI, the intrathecal injection of gadobutrol was carried out by an interventional neuroradiologist. Correct position of the syringe tip in the subarachnoid space was verified by CSF backflow from the puncture needle. Gadobutrol was administered in a dose of 0.5 mmol (0.5 ml of 1.0 mmol/ml gadobutrol; Gadovist®, Bayer Pharma AG). After injection, the patient was instructed to rotate once around the body axis on the table. Study participants were kept flat until the last MRI acquisition Day 1 and allowed to move freely thereafter.

Gadobutrol increases the T_1 relaxation of water and hence results in higher signal intensity at the image greyscale when present in CSF or brain tissue. The T_1 signal intensity thus provides a semiquantitative measure of the tracer concentration.

Image analysis

The multiple MRI acquisitions were aligned, and we used FreeSurfer software (version 6.0) (http://surfer.nmr.mgh.harvard.edu/) for segmentation, parcellation and registration/alignment of the longitudinal data. The segmentation and parcellation acquired from Freesurfer were used to investigate the increase of T_1 intensity caused by the CSF tracer. The methods are documented in a review (Fischl, 2012). Non-brain tissue is removed using a hybrid watershed/surface deformation procedure (Segonne *et al.*, 2004), followed by automated Talairach transformation and segmentation of the subcortical white matter, and deep grey matter structures (including hippocampus, amygdala, caudate, putamen and ventricles) (Fischl *et al.*, 2002, 2004). The magnetic resonance images of each patient were used to create a median template registered to the baseline, as previously described (Reuter *et al.*, 2012). Hence, for each patient the magnetic resonance images were registered to the corresponding template using a rigid transformation (Reuter *et al.*, 2012). The registrations were subsequently checked manually by V.V. to correct for any registration errors.

For image analysis at the group level, a template was created using scans of all subjects for all time points. The template was created with 'mri_robust_template' and segmented with 'recon-all' in FreeSurfer; thereafter all segmentations were visually inspected for possible errors, not disclosing any structural differences between the templates of the two groups. With the aligned image data and the template segmentation, a median image of

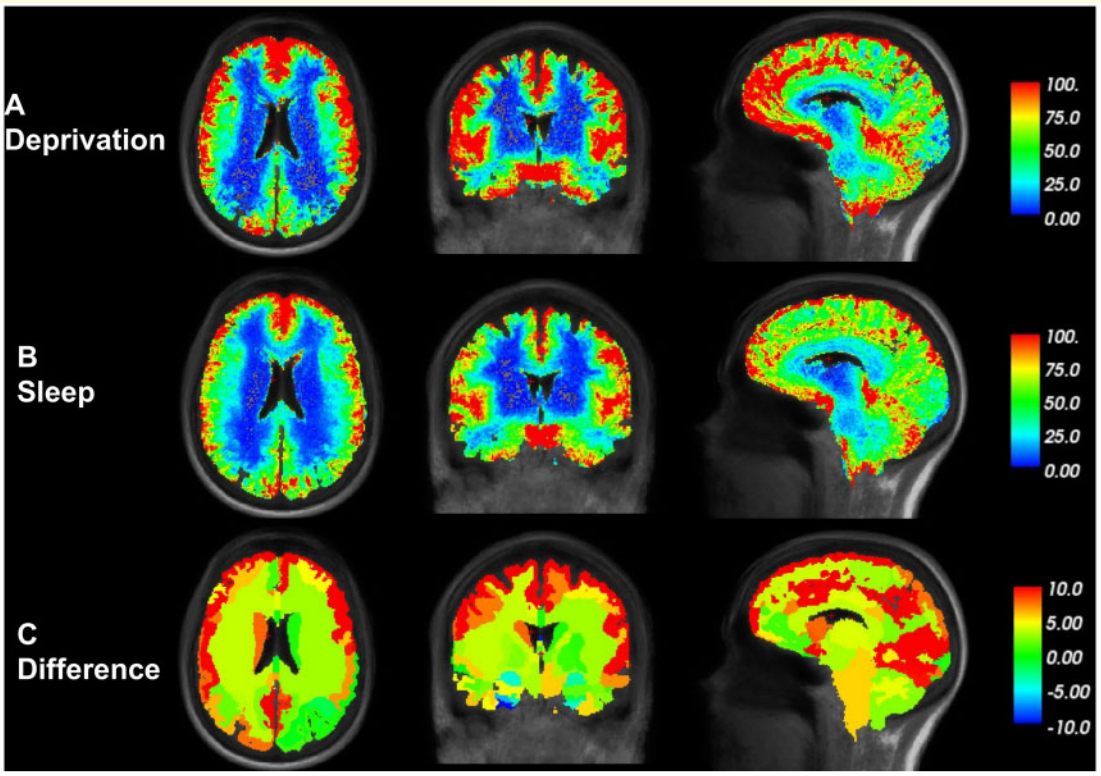

Figure 1 ***In vivo*** **brain imaging shows that one night of total sleep deprivation reduces molecular clearance from the human brain.** The images present tracer enrichment within brain tissue, while tracer in CSF spaces have been subtracted. Tracer enrichment in brain tissue is expressed by MRI signal percentage increase from baseline, and shown on the colour scale. (**A**) The tracer-dependent signal increase in brain is presented on average for the cohort undergoing total sleep deprivation from Day 1 to Day 2 (n = 7; sleep deprivation group). Axial (*left*), coronal (*middle*) and sagittal (*right*) MRI scans are presented with the percentage signal increase from baseline, indicated by the colour scale. (**B**) The average percentage signal increase from tracer enrichment is presented for the cohort allowed to sleep freely from Day 1 to Day 2 (n = 17; sleep group). (**C**) The percentage difference in signal increase between the cohorts (sleep deprivation minus sleep groups) is shown. The colour scale shows that tracer levels in the brain tissue were higher after sleep deprivation compared to sleeping subjects. The red colour represents areas with the highest tracer levels. In particular, clearance of tracer after sleep deprivation was most impaired in frontal, temporal, parietal and cingulate cortical areas.

each group was created at given time points. For each group, the relative increase in intensity from the last time point of Day 1 to Day 2 was computed. The differential map shown in Fig. 1 was created by subtracting the median relative increase of the sleep deprivation group from the median relative increase in the sleep group. Finally, for the visualization, each segmented region was assigned the median value of the differential map in the respective region.

Normalization of T_1 signal

To adjust for changes in the greyscale between MRI scans, the T_1 signal unit for each time point was divided by the T_1 signal unit of a reference region of interest for the respective time point. The reference region of interest was placed within the posterior part of the orbit. The placement of the reference region of interest is illustrated in Supplementary Fig. 2. The ratio represents the 'normalized T_1 signal units' and corrects for any baseline changes of image greyscale due to automatic image scaling.

Statistical analyses

Continuous data were presented as mean (standard deviation, SD) or mean (standard error, SE), as appropriate. Normal distribution of data was assessed in both groups. We estimated from the image analysis the mean and standard error at 0 (pre-contrast), 0–1.5 h, 1.5–3 h, 4.5–7 h, 24 h, 48 h, and at 4 weeks follow-up. The repeated measurements were assessed with linear mixed models by maximum likelihood estimation using a subject-specific random intercept and including for main regions parenchyma of brain a nested random effect of brain segments. Using estimated marginal mean from the statistical model, we tested the difference between the sleep deprivation group and sleep group at the different points of follow-up.

The statistical analysis was performed using SPSS version 26 (IBM Corporation, Armonk, NY) and Stata/SE 15.0 (StataCrop LLC, College Station, TX). Statistical significance was accepted at the 0.05 level (two-tailed).

Data availability

The data presented in this work are available upon request.

Results

Patients

Seven individuals underwent total sleep deprivation from Day 1 to Day 2 (sleep deprivation group), and 17 individuals slept from Day 1 to Day 2 (sleep group). The two groups were similar with regard to age, gender, body mass index (BMI) and tentative diagnosis (Table 1). While individuals in the sleep deprivation group had no sleep from Day 1 to 2, individuals in the sleep group had 6.4 ± 1.9 h sleep from Day 1 to 2. Subjective sleep quality was 'deep' in 10 individuals, 'medium' in five and 'light' in two subjects (Table 1).

Tracer enrichment within the CSF space

Following intrathecal injection, the tracer distributed in the subarachnoid spaces intracranially, primarily in the basal cisterns and along the major artery trunks (anterior, middle and posterior cerebral arteries) at the brain surface, allowing for free mixing with CSF (Supplementary Video 1). A region of interest was placed manually in each image volume at the level of the cisterna magna to assess tracer enrichment in CSF spaces (Supplementary Fig. 3). Tracer enrichment of the intracranial CSF spaces was confirmed in all study participants, with no difference in enrichment level between the sleep deprivation and sleep groups throughout the imaging period (Supplementary Table 1).

The normalized MRI T_1 signal within the CSF, indicative of tracer enrichment in CSF spaces, was comparable between the sleep and sleep deprivation groups (Supplementary Table 1). After intrathecal injection, the CSF tracer enriched the subarachnoid CSF spaces; at 4.5–7 h the normalized T_1 signal was significantly increased in the sleep ($P < 0.001$) and sleep deprivation ($P = 0.007$) groups, but with no differences between groups.

Tracer enrichment within brain tissue

The tracer gadobutrol enriches brain tissue from the surface. Notably, tracer enhancement in brain tissue represents molecular movement in the extravascular spaces (i.e. perivascular and interstitial spaces). Tracer enriched in all main brain regions (Fig. 2). The time course of enrichment of tracer within the extravascular compartment of the brain is illustrated for one individual of the sleep group in Fig. 3 (see also Supplementary Video 2). Furthermore, Supplementary Table 2 demonstrates—for a wide range of brain locations—a highly significant change in T_1 signal after 4.5–7 h, which indicates extravascular enrichment of CSF tracer in brain tissue. As can be seen, the CSF tracer passes to nearly all examined regions of the brain. For the majority of brain subregions, there were no differences in tracer enrichment between groups at 4.5–7 h, except for a few locations.

Increased amount of CSF tracer in brain after total sleep deprivation

Our main question was whether tracer levels within brain parenchyma differed after one night of total sleep deprivation. Elevated levels of tracer on a declining enhancement curve would here be defined as reduced molecular clearance from the brain. After one night, both groups still displayed substantial tracer enrichment within the brain, but the tracer enrichment was higher in the sleep deprivation group compared to the sleep group (Fig. 1). After 24 h, tracer levels were similar within the CSF spaces of the two groups (Fig. 4A), demonstrating that clearance of tracer from CSF spaces was not affected by sleep deprivation. On the other hand, in the sleep deprivation group there were significantly increased tracer levels within the cerebral cortex (Fig. 4B) and cerebral white matter (Fig. 4C), as sign of reduced tracer clearance. Moreover, the impaired molecular clearance from sleep deprivation was evident in structures typically considered part of, or closely linked to, the limbic system, such as amygdala, hippocampus, nucleus accumbens, prefrontal cortex, insula and cingulum (Fig. 5 and Supplementary Table 3).

Between the 24 h to 48 h time points, no sleep restrictions were given to any of the groups. Still, the tracer levels in the brain tissue remained elevated at 48 h in the sleep deprivation group (Figs 1, 5 and Supplementary Table 3), suggesting a lost night of sleep is not immediately compensated for by a 1-day recovery period of unrestricted sleep.

Forty-eight hours after sleep deprivation, differences between groups were noted within numerous white matter regions (Supplementary Table 3). We cannot conclude whether this was primarily an effect of the sleep intervention, or secondary to different tracer levels in the cortex above. Enrichment of white matter was highly associated with enrichment of the adjacent grey matter of cerebral cortex after 4–5–7 h (Fig. 6A), 24 h (Fig. 6B) and 48 h (Fig. 6C). Thus, the events that we observed within the white matter seem dependent on the enrichment of grey matter.

No residual tracer in CSF after 4 weeks

After 4 weeks, there were no differences in normalized T_1 signal as compared to before intrathecal CSF tracer administration in any of the groups (Supplementary Table 4), i.e. no signs of remaining contrast agent in CSF or brain tissue in any of the groups.

Discussion

This study provides the first *in vivo* evidence that sleep deprivation results in impaired molecular clearance from the human brain and that one night clearance failure may not be compensated by subsequent sleep.

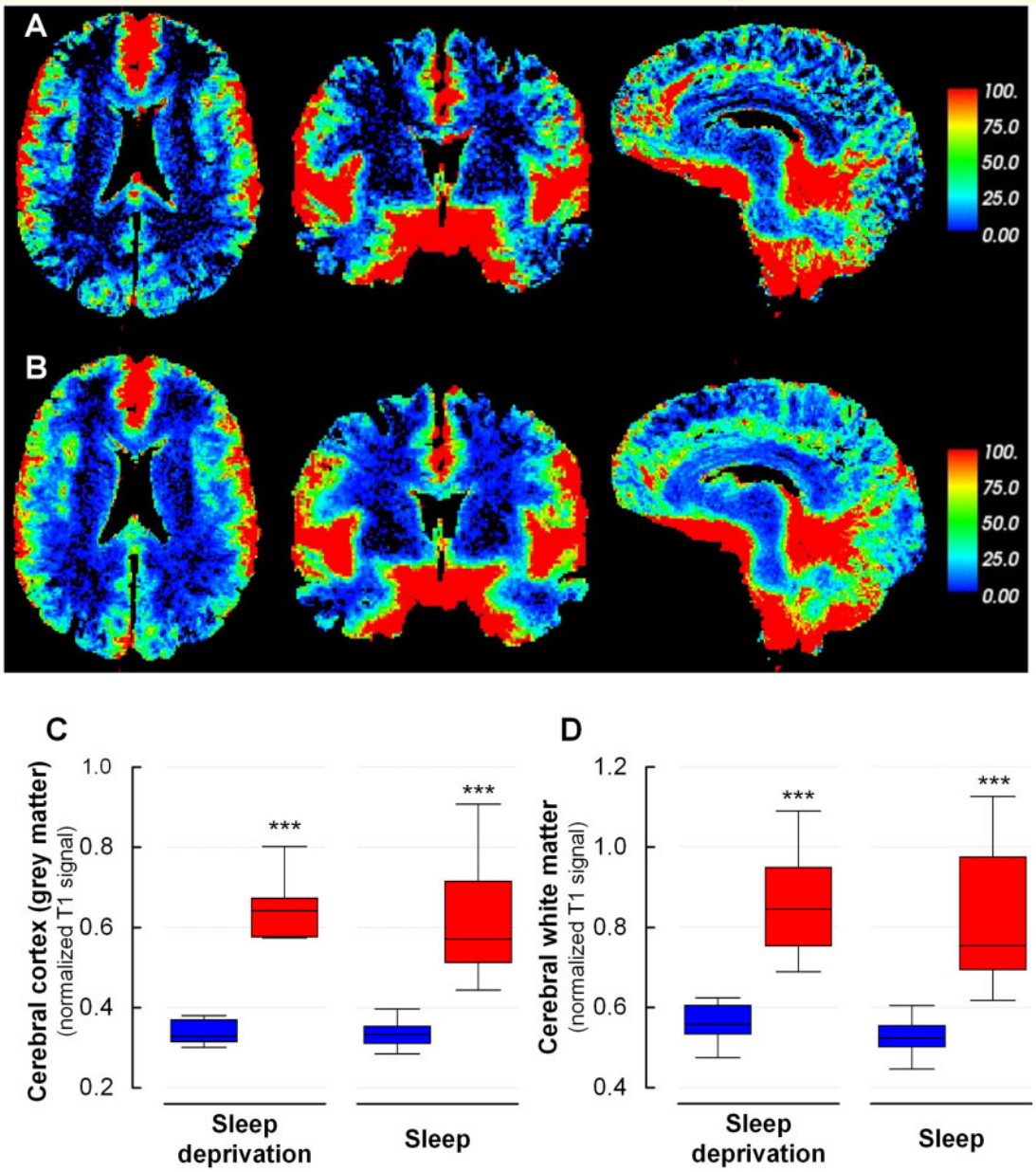

Figure 2 The CSF tracer enriches the extravascular compartment of the brain centripetally. The images present tracer enrichment within brain tissue, while tracer in CSF spaces has been subtracted. The degree of tracer enrichment in brain tissue is expressed by the MRI signal increase, and shown by the colour scale. Tracer enrichment was brain-wide and comparable between the groups at the last MRI scan on Day 1 preceding sleep deprivation. The average percentage signal increase in the brain after 4.5–7 h is shown for all subjects within (**A**) the sleep deprivation (n = 7) and (**B**) sleep (n = 17) groups. For both groups, it should be noted that tracer enrichment occurred in a centripetal pattern, and concentrated particularly in brain regions adjacent to large artery trunks at the surface. The coronal section (*middle*) shows enhancement in the medial temporal lobe close to the circle of Willis and posterior cerebral arteries, in the cingulum adjacent to the location of the anterior cerebral arteries in the anterior interhemispheric fissure, and around the Sylvian fissure, where the middle cerebral artery trunks reside. Compared to before tracer administration (blue), the normalized T_1 signal had increased markedly after 4.5–7 h (red) within grey matter of cerebral cortex (**C**) and cerebral white matter (**D**).

The two groups were comparable with regard to age, gender and tentative diagnosis. The level of tracer in CSF space was similar throughout the study period and should therefore have not confounded different levels of tracer in brain tissue at any time point. In the end, we therefore regard the sleep intervention to be the only factor accompanied with impaired clearance of tracer from the brain in the sleep deprivation group. Total sleep deprivation was verified in the sleep deprivation group by the patient, the attending family members and nursing staff within the neurosurgical ward, where the participants stayed.

It was established many years ago that acute sleep deprivation negatively affects a wide range of cognitive functions,

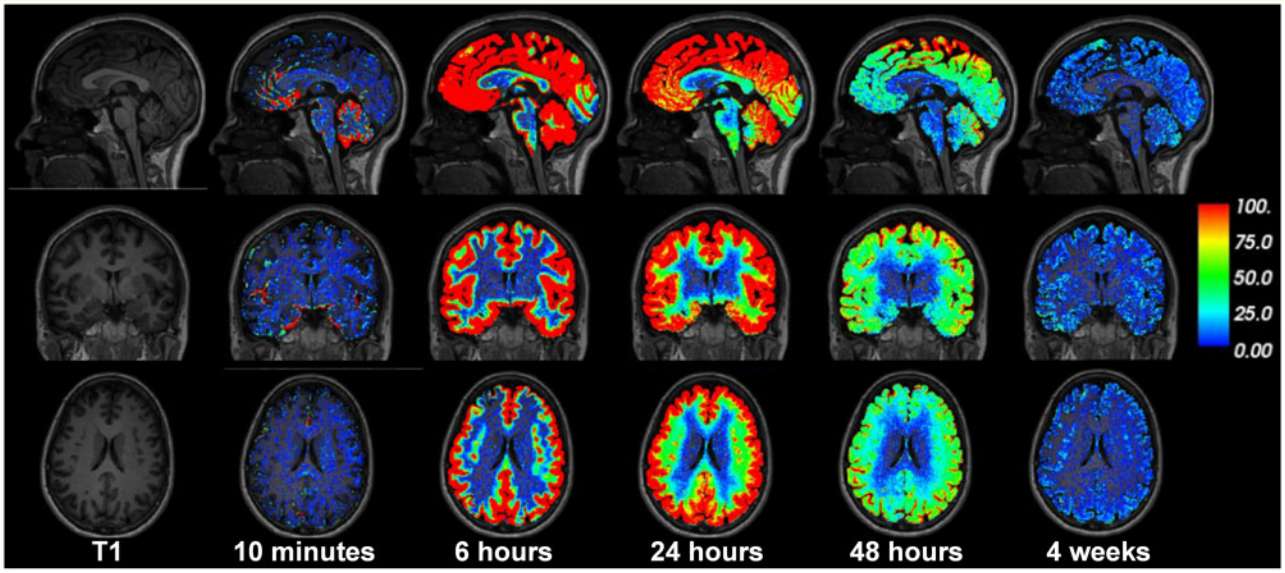

Figure 3 Brain-wide CSF tracer enrichment over time for one individual from the sleep group. The images present tracer enrichment within brain tissue, while tracer in CSF spaces have been subtracted. Standardized T_1-weighted MRI was obtained at different time points before and after intrathecal gadobutrol, serving as CSF tracer. Percentage T_1 signal increase from tracer enrichment in brain tissue is illustrated by the colour scale. Positive and negative image noise are coded blue or transparent, respectively. Because of a lower signal-to-noise ratio, noise is most apparent in images from time points with no tracer enrichment in the brain. After 4 weeks, there was no sign of residual tracer in brain tissue (Supplementary Table 4).

including memory, learning, attention, emotional reactivity (Lim and Dinges, 2010). Sleep deprivation for days and weeks may even be fatal (Rechtschaffen *et al.*, 1983; Shaw *et al.*, 2002). A progressive type of insomnia has been described in humans (familial or sporadic) that results in aggravating loss of sleep and eventually dementia and death (Montagna *et al.*, 2003). Chronic sleep deprivation has even emerged as a major risk factor for Alzheimer's disease and neurodegeneration in general (Moran *et al.*, 2005). An ordinary explanation for sleep disturbance in dementia has been dementia-related death of neurons involved in sleep function including the hypothalamic suprachiasmatic nucleus (Swaab *et al.*, 1985). This region is involved in circadian rhythm as the suprachiasmatic senses light via the retinohypothalamic tract (Moore and Eichler, 1972; Berson *et al.*, 2002). Over the last few years, more attention has shifted towards the role of sleep in clearance of waste products, such as amyloid-β (Xie *et al.*, 2013).

Whether or not sleep affects molecular clearance from the human brain has never been proven. The previous *in vivo* observations from mice (Xie *et al.*, 2013), using two-photon microscopy, assessed events occurring <0.5 mm below the cortical surface after removal of the scull bone, which is known to affect the pulsatile nature of the brain. Our brain-wide observations show that sleep deprivation affects clearance even within deep parts of the brain, and that the effect of sleep deprivation is protracted (peak after 24–48 h). In particular, differences were evident within the limbic system, which represents the phylogenetically oldest part of the cerebral cortex (allocortex) with resemblance between humans and lower species. The proximity of these regions to large artery trunks at the brain surface may suggest that the arterial pulsation by their propagation to CSF is an important driving force for nearby parenchymal tracer enrichment, as shown previously (Ringstad *et al.*, 2018). Limbic structures also correspond to some degree with areas susceptible to consecutive tau propagation in Alzheimer's disease (Braak and Braak, 1991).

The mechanisms by which molecules are cleared from the brain are currently extensively debated, not least facilitated by the description of a glymphatic system in rodent brain (Iliff *et al.*, 2012). The glymphatic system is a paravascular transport route for convective motion of fluids and solutes along the arterial tree penetrating the brain, via interstitial tissue and finally along the venous vessels, driven by artery pulsations and being dependent on the water channel aquaporin 4 (AQP4) at astrocytic endfeet surrounding the cerebral vasculature (Iliff *et al.*, 2012). The concept has been criticized regarding the role of AQP4 (Smith and Verkman, 2018), mechanisms behind molecular transport in interstitial tissue (Holter *et al.*, 2017), as well as studies indicating that paravascular transport of solutes such as amyloid-β occur retrograde along the basement membrane and muscular wall of arteries towards lymphatic vessels (Weller *et al.*, 2007; Bakker *et al.*, 2016). Due to the limited resolution of MRI (1 mm), we cannot conclude with certainty about the exact passage route of the tracer within brain tissue. Our observations utilizing intrathecal contrast-enhanced MRI may,

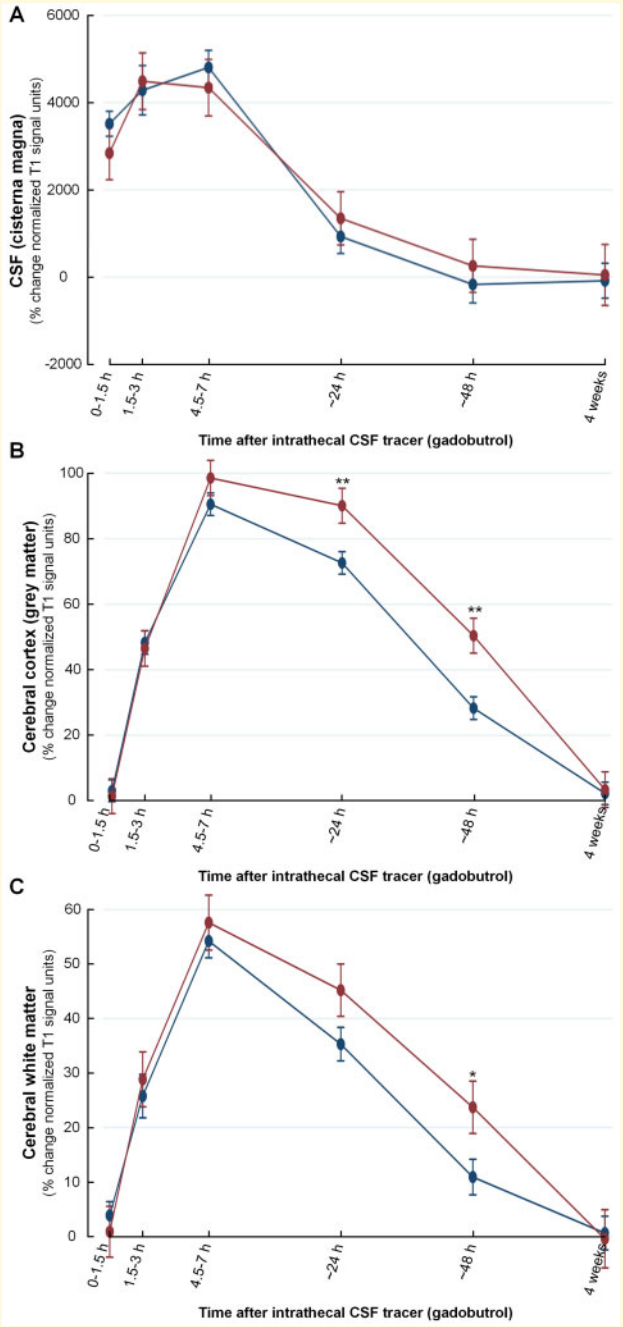

Figure 4 One night of total sleep deprivation reduces clearance of tracer from the brain that lasts even after another night of sleep. Trend plots of percentage change in signal unit ratio, indicative of tracer enrichment within brain tissue, are presented for different regions, including (**A**) CSF space at the craniocervical junction, (**B**) cerebral cortex (grey matter), and (**C**) cerebral white matter. While tracer levels were similar between the groups in the CSF space (**A**), there were significant differences between the sleep deprivation (red) and sleep (blue) groups in grey matter of the cerebral cortex (**B**) and white matter (**C**) at 24 h (i.e. after sleep intervention) and at 48 h (both groups were allowed to sleep freely from 24 to 48 h). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Trend plots are presented with mean ± SE from linear mixed models.

however, provide support to the glymphatic concept, namely the antegrade transport of CSF tracer along arteries (Ringstad *et al.*, 2017), centripetal enrichment of brain tissue from outside cerebral cortex of a tracer strictly confined outside vessels due to the blood–brain barrier (Ringstad *et al.*, 2018), and evidence for transport faster than extracellular diffusion in the observed tracer movement (Valnes *et al.*, 2020). The present observations of sleep-dependent tracer enrichment within brain tissue may also support the glymphatic concept, given that sleep-dependent molecular clearance is a key part of the glymphatic concept (Xie *et al.*, 2013).

Independent the mechanisms behind brain molecular clearance, the dural lymphatic vessels seem to represent the main efflux route for solutes from intracranial CSF spaces (Louveau *et al.*, 2017). In humans, parasagittal dura serves as a bridging link between subarachnoid CSF spaces and the dural lymphatic vessels (Ringstad and Eide, 2020). It was recently shown that lymphatic drainage was faster in awake than anaesthetized mice (Ma *et al.*, 2019), which might indicate that glymphatic clearance of waste solutes occurs during sleep while during the awake state lymphatic efflux to extracranial lymph nodes is enhanced. On the other hand, we found no evidence that sleep deprivation affects clearance of tracer from CSF spaces (Fig. 4A and Supplementary Table 1), suggesting that lymphatic molecular efflux routes are less affected by sleep in humans. To what extent sleep may be instrumental for lymphatic molecular efflux rate needs to be addressed in future studies.

Some study limitations should be noted. Here, we added a tracer to CSF that subsequently enriched the brain extravascular compartment centripetally, from outside to inside. There is an agreement that substances are cleared centrifugally under physiological conditions (Weller *et al.*, 2007; Iliff *et al.*, 2012). In its clearance phase from the brain, the tracer is thus expected to move against a concentration gradient, unlike endogenous byproducts of brain metabolism. Furthermore, the limited temporal resolution of MRI scans did not allow us to precisely assess the timing of peak brain enrichment, or the exact time point when groups differed most. Finally, the lack of EEG registrations during sleep deprivation renders us unable to exclude the possibility of micro-sleep. Any of these limitations may therefore have made us underestimate the impact of sleep on molecular clearance.

The present findings are in line with the hypothesis that parenchymal CSF tracer movement is governed by the sleep-wake cycle, rather than the circadian rhythm *per se*. In mice, adrenergic antagonists increased CSF tracer influx to parenchyma comparably with influx during sleep or anaesthesia (Xie *et al.*, 2013). The authors proposed that locus coeruleus adrenergic-mediated shrinkage of cell volume reduced resistance to diffusion or bulk (convective) flow during sleep might enhance clearance of waste solutes such as amyloid-β from brain parenchyma. It is well established that locus coeruleus-derived noradrenergic signalling is instrumental in regulating arousal and awake state (Carter *et al.*, 2010;

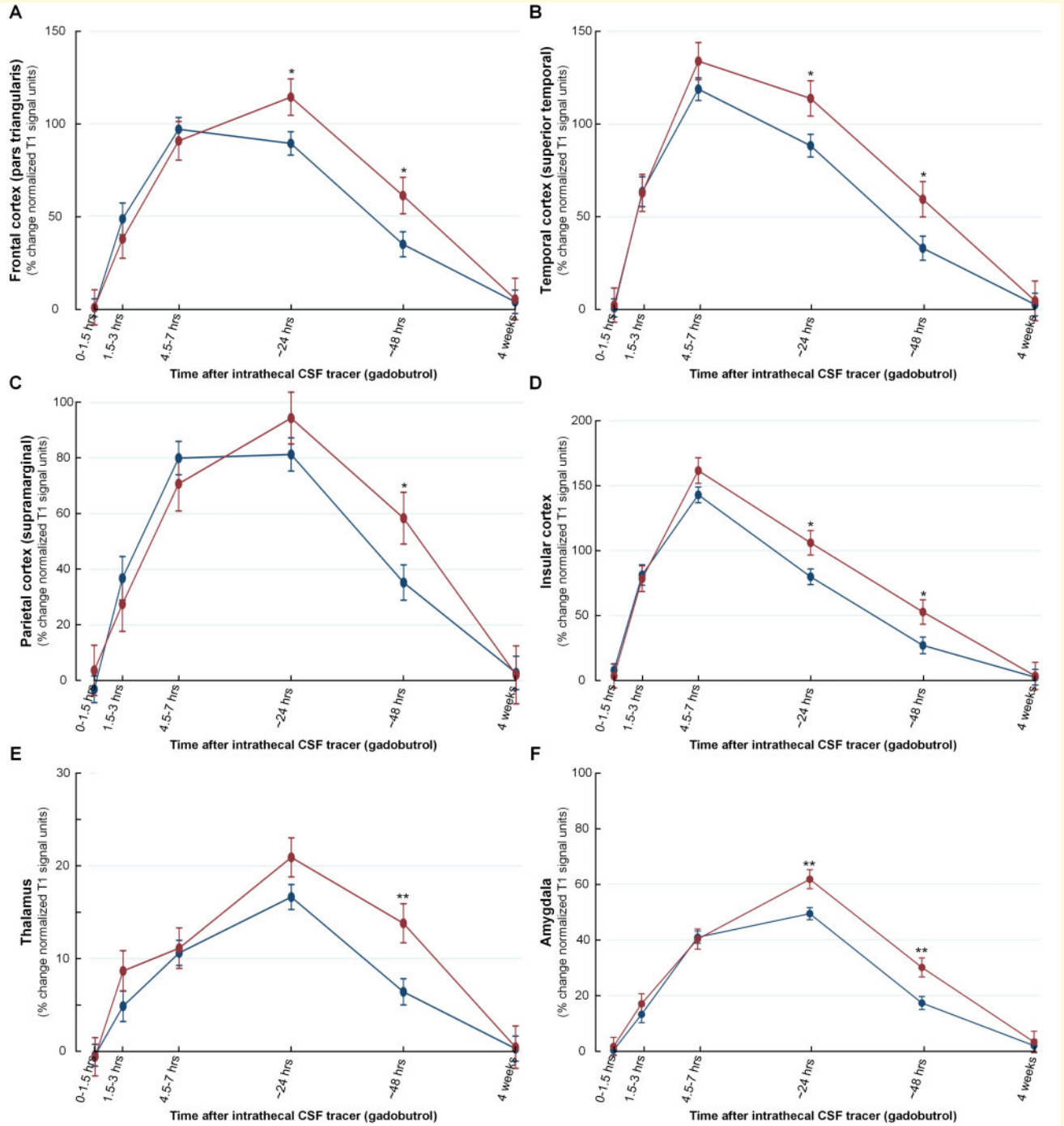

Figure 5 Increased CSF tracer levels in a selection of brain regions indicative of reduced molecular clearance after total sleep deprivation. Delayed molecular clearance after sleep deprivation is exemplified by trend plots of percentage change in signal unit ratio from a selection of brain regions, including (**A**) gyrus triangularis in frontal cortex, (**B**) superior temporal gyrus in temporal cortex, (**C**) supramarginal gyrus in parietal cortex, (**D**) insular cortex, (**E**) thalamus, and (**F**) amygdala. Significant differences between the sleep group (blue) and sleep deprivation group (red) were determined at each individual time point. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Trend plots are presented with mean ± SE.

Constantinople and Bruno, 2011). The neurons from locus coeruleus project to the basal forebrain, pre-optic area, hypothalamus, thalamus and cortex (Akeju *et al.*, 2014; Purdon *et al.*, 2015). Here we noted evident clearance failure in these same regions following sleep deprivation. In Alzheimer's disease, there is degeneration of locus coeruleus and increased noradrenergic tone, reflected by increased CSF concentrations of norepinephrine (Elrod *et al.*, 1997; Szot

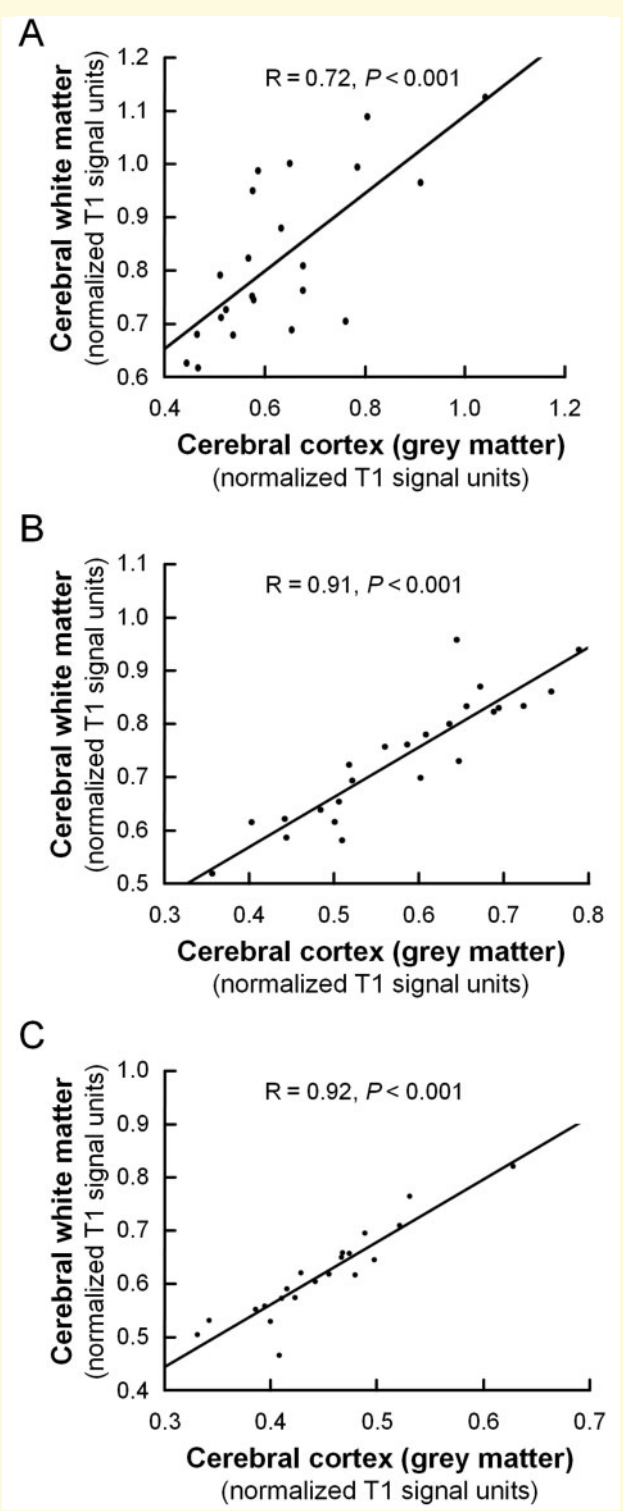

Figure 6 Association between tracer enrichment in cerebral cortex and white matter. The association between tracer enrichment (normalized T_1 signal increase) within the cerebral cortex and cerebral white matter at different time points, namely (**A**) 4.5–7 h, (**B**) 24 h and (**C**) 48 h after intrathecal CSF tracer administration. For all time points, there was a highly significant correlation between tracer enrichment within the cortical grey matter and cerebral white matter. The fit line and the Pearson correlation coefficient (R) with significance *P*-value is presented for each plot.

et al., 2006, 2007). In this regard, it should be mentioned that the anaesthetic agent dexmedetomidine is an α2 agonist that hyperpolarizes locus coeruleus neurons and decreases firing rate and norepinephrine release (Jorm and Stamford, 1993), and induces a state comparable to stage II sleep with an increase in slow-wave activity (0.5 to 3.5 Hz) (Purdon *et al.*, 2015). This compound was found to enhance glymphatic clearance in rats (Benveniste *et al.*, 2017), and to enhance delivery of intrathecal drugs to the brain in rats (Lilius *et al.*, 2019). On this background, manipulation of noradrenergic tone may emerge as a method for modifying extravascular (interstitial and perivascular) molecular transport in the brain. The extracellular space constitutes ~20% of the overall brain volume (Sykova and Nicholson, 2008), and the cortical interstitial volume fraction was 13–15% in mice in the awake state compared to 22–24% in the sleeping or anaesthetized state (Xie *et al.*, 2013).

Conclusions

In conclusion, we provide *in vivo* evidence that one night of total sleep deprivation impairs molecular clearance from the human brain, an effect not compensated for by another night's sleep. The results support the hypothesis that the interstitial space increases in the sleeping human brain, as previously demonstrated in rodents. As such, the observations have implications for understanding the impact of disturbed sleep in the evolvement of neurodegenerative disease, and may point to avenues for enhancing endogenous molecular transport and delivery of intrathecal drugs.

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Competing interests

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Supplementary material

Supplementary material is available at *Brain* online.

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