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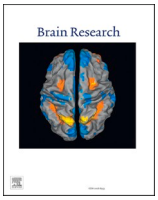

# Cerebrospinal fluid egress to human parasagittal dura and the impact of sleep deprivation

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ABSTRACT

Emerging evidence suggests that the glymphatic system and meningeal lymphatic vessels are instrumental for clearance of toxic metabolites from the brain. Animal and human studies suggest that glymphatic circulation is up-regulated during sleep. Meningeal lymphatic clearance may be more efficient in the wake state, as shown in rodents. We have previously shown clearance of cerebrospinal fluid directly from the subarachnoid space to the parasagittal dura, which harbors meningeal lymphatic vessels. Hence, assessing molecular clearance from parasagittal dura provides an opportunity to decipher the role of sleep/sleep deprivation in human lymphatic clearance function. In this study, we applied magnetic resonance imaging to explore whether sleep deprivation modifies molecular clearance from human parasagittal dura, utilizing an intrathecal magnetic resonance imaging contrast agent as tracer. We hypothesized that tracer enhancement in parasagittal dura would differ after sleep deprivation. One group of individuals (n = 7) underwent one night's total sleep deprivation while a control group (n = 9) was allowed unrestricted sleep. There were no sleep restrictions after the 24-hour time point. After one night of sleep deprivation (at 24 h), we found neither evidence for altered tracer enrichment in the parasagittal dura, nor after a day of unrestricted sleep (at 48 h). The hypothesis of altered molecular egress to parasagittal dura after sleep deprivation was not supported by our data. Further studies are required to determine the role of sleep for molecular clearance from cerebrospinal fluid to meningeal lymphatic vessels in humans.

## 1. Introduction

In 2015, meningeal lymphatic vessels were (re)discovered within the dura mater close to the dural sinuses and found to drain molecules from CSF, and hence from the brain (Aspelund et al., 2015; Louveau et al., 2015). Emerging evidence suggests that impaired dural lymphatic clearance of toxic metabolites [e.g. amyloid-β, hyper-phosphorylated tau protein (tau) and α-synuclein] is instrumental for evolvement of neurodegenerative disease and dementia (e.g. Alzheimer and Parkinson diseases) (Da Mesquita et al., 2018; Ding et al., 2021; Louveau et al., 2016; Louveau et al., 2017; Patel et al., 2019; Sweeney and Zlokovic, 2018; Wang et al., 2019; Zou et al., 2019). Impaired lymphatic clearance may as well aggravate consequences of multiple sclerosis (Louveau et al., 2016), subdural hematoma (Liu et al., 2020), stroke and subarachnoid hemorrhage (Chen et al., 2020), traumatic head injury (Bolte et al., 2020) and malignant disease (Hu et al., 2020; Kanamori and Kipnis, 2020). The meningeal lymphatic system seems to work in concert with the glymphatic system (Louveau et al., 2017), which is a perivascular system for transport of fluid and solutes within the central nervous system (CNS) (Iliff et al., 2012).

The glymphatic/meningeal lymphatic function may be under control of the sleep-wake state. Experimental evidence from mice demonstrated marked upregulation of glymphatic function during sleep (Xie et al., 2013), suggesting that the glymphatic system is primarily active during sleep. Supporting this concept, we recently provided in vivo evidence that sleep deprivation impairs glymphatic molecular clearance from human brain (Eide et al., 2021). Lymphatic molecular egress from CSF has also been found markedly enhanced in wake compared to anesthetized mice, and was accompanied by reduced paravascular molecular influx to brain (Ma et al., 2019). Another study in mice demonstrated that CSF drainage to cervical lymph nodes is under circadian control with peak clearance during the awake state (Hablitz et al., 2020). An impact of the sleep-wake state on meningeal lymphatic function would be highly significant since dural lymphatics seem to represent a final

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common pathway for clearance of toxic molecules from the CSF (Ma et al., 2017).

The parasagittal dura harbors true lymphatic vessels and was recently found to represent an important immune hub for presentation of brain-derived antigen to trafficking peripheral T-cells in rodents (Rustenhoven et al., 2021). In humans, direct trans-arachnoid egress of CSF tracer to parasagittal dura has previously been demonstrated in vivo (Ringstad and Eide, 2020), rendering for direct molecular egress from the glymphatic system via CSF to meningeal lymphatic vessels, where tight junctions are absent (Louveau et al., 2015). Furthermore, molecular passage via the cribriform plate to nasal mucosa seems to be of minor importance in humans (Melin et al., 2020) in contrast to other species, e.g. rodents (Johnston et al., 2004), suggesting that species differences can be important to assess.

The aim of the presents study was therefore to examine whether CSF tracer enhancement in human parasagittal dura is affected by sleep deprivation. We hypothesized that sleep deprivation would facilitate meningeal lymphatic drainage. Therefore, we compared a cohort of individuals undergoing one night's total sleep deprivation with a cohort allowed unrestricted sleep and further followed both groups the following 24 h of unrestricted sleep. CSF tracer enrichment in parasagittal dura and nearby subarachnoid CSF was semi-quantified using intrathecal contrast enhanced magnetic resonance imaging, with the contrast agent serving as tracer molecule.

## 2. Results

### 2.1. Patients

The study included 16 consecutive patients undergoing work-up of various CSF disorders (Table 1). The time from intrathecal injection of gadobutrol to first detection in foramen magnum by visual inspection was 10.9 ± 10.9 min in the Sleep Deprivation group (n = 7) and 8.4 ± 3.1 min in the Sleep group (n = 9; P = 0.54, independent samples *t*-test). Tracer enrichment in parasagittal dura is visualized as change in T1-BB signal, as shown in Fig. 1. Three-dimensional representations of the parasagittal dura and tracer enrichment in nearby CSF is shown in Fig. 2.

**Table 1**
Information about the Sleep and Sleep Deprivation cohorts.

| | Sleep cohort | Sleep Deprivation cohort | Significance |
|---|---|---|---|
| N | 9 | 7 | |
| Sex (F/M) | 8/1 | 6/1 | ns |
| Age (years) | 36.1 ± 13.0 | 44.7 ± 15.7 | ns |
| BMI (kg/m$^2$) | 29.5 ± 7.2 | 26.2 ± 3.7 | ns |
| Tentative diagnosis | | | |
| Non-verified CSF leakage | 2 (22.2%) | 2 (28.6%) | ns |
| Pineal cyst (non-surgery) | 3 (33.3%) | 1 (14.3%) | ns |
| Arachnoid cyst (non-surgery) | 0 | 1 (14.3%) | ns |
| Hydrocephalus (non-surgery) | 1 (11.1%) | 0 | ns |
| IIH (non-surgery) | 2 (22.2%) | 1 (14.3%) | ns |
| IIH (surgery) | 1 (11.1%) | 2 (28.6%) | ns |
| Sleep Day 1 to 2 | | | |
| Total sleep deprivation | – | 7 | |
| Hours of sleep | 6.4 ± 2.0 | 0 | |
| Subjective sleep quality Day 1 to 2 | | | |
| Light | 1 | | |
| Medium | 4 | | |
| Deep | 4 | | |

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 Chi-square test for categorical data. Ns: Non-significant. The subjective sleep quality from Day 1 to Day 2 is indicated.

### 2.2. Tracer enrichment of parasagittal dura relies on tracer availability in CSF

Tracer enrichment data from brain and CSF for the study cohort has been reported previously (Eide et al., 2021). We here only included patients with MRI T1-BB sequences.

A high degree of correlation between percentage change of tracer level in subarachnoid CSF space and parasagittal dura was found for both groups (Table 2). Therefore, in both groups the enrichment of tracer within parasagittal dura was highly dependent on presence of tracer in adjacent dura.

### 2.3. Tracer enrichment in parasagittal dura is not affected by sleep deprivation

The tracer molecule enriched the subarachnoid space nearby parasagittal dura to a similar degree in both the Sleep and Sleep Deprivation groups (Fig. 3a), ensuring comparable availability of tracer within the CSF spaces in both groups at 24 and 48 h. Table 3 presents the normalized T1-BB signal over time for CSF and parasagittal dura, respectively.

Enrichment of tracer within the parasagittal dura was similar in the Sleep- and Sleep Deprivation groups, respectively, at any time point, including after 24 and 48 h (Fig. 3b). Even though the change in tracer enrichment within parasagittal dura from six to 24 h seemed to be more evident in the Derivation than Sleep group, this was neither significant for percentage change (P = 0.23), nor for change in signal unit ratios (P = 0.30; data not shown).

## 3. Discussion

The presently reported observations showed that enrichment of tracer in parasagittal dura and nearby subarachnoid CSF was unaffected by one-night's total sleep deprivation. These findings extend our recently published observations of impaired molecular clearance from the brain following sleep deprivation (Eide et al., 2021), by suggesting that the sleep deprivation has a minor impact on CSF clearance to and from human parasagittal dura, an important hub for brain lymphatic drainage and immune surveillance (see illustration Fig. 4).

The present observations support previous observations (Ringstad and Eide, 2020), that meningeal lymphatic egress in this particular region depends on level of molecular enrichment within the nearby CSF, suggesting passive transport along a concentration gradient. Whether or not an active energy-dependent process still can be present needs to be determined in future studies.

It is well established that both acute and chronic sleep deprivation is negative for man. Acute sleep deprivation impairs cognitive function (Lim and Dinges, 2010), and sleep deprivation for days or weeks may be fatal (Rechtschaffen et al., 1983; Shaw et al., 2002). In humans, a progressive type of insomnia may be fatal (Montagna et al., 2003). Chronic sleep deprivation, on the other hand, is a risk factor for neurodegeneration and Alzheimer's disease (Moran et al., 2005). The underlying mechanisms have been discussed, though several lines of evidence point at cerebral accumulation of toxic metabolites as a mechanism behind negative effects of sleep deprivation. Accordingly, a mice AD model revealed increased interstitial levels of amyloid-β (Kang et al., 2009) and tau (Holth et al., 2019) after acute sleep deprivation, and increased amyloid-β plaque formation after chronic sleep deprivation (Kang et al., 2009). With regard to findings in humans, an amyloid-β PET study of 20 healthy individuals showed that one night of total sleep deprivation increased parenchymal amyloid-β burden by 5% in (Shokri-Kojori et al., 2018).

The arachnoid was previously thought to constitute an impermeable barrier layer between the CSF and dura mater (Coles et al., 2017; Ma et al., 2017; Nabeshima et al., 1975). Contrary to previous ideas, the in vivo observations of direct passage of tracer from subarachnoid CSF to

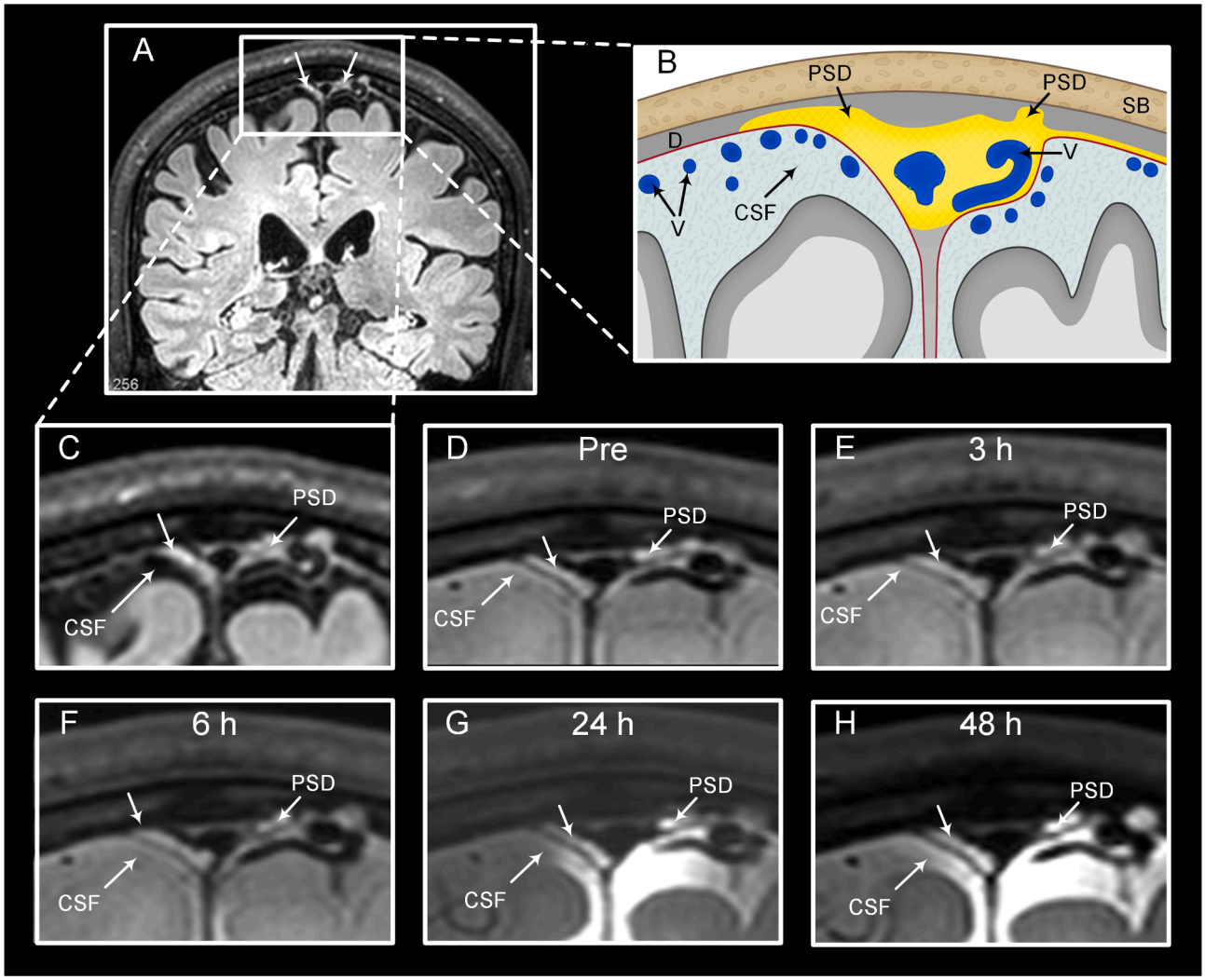


**Fig. 1.** Direct molecular egress of tracer molecule from subarachnoid CSF space to parasagittal dura in human subject. (A and C) Coronal T2-FLAIR MRI demonstrates the parasagittal dura (PSD) with high signal (arrows) in one patient of the Sleep cohort. (B) A cartoon of the section from T2-FLAIR illustrates PSD with yellow color; meningeal lymphatic vessels within PSD are not visualized on MRI due to their size far below the image resolution (1 mm). Also note the vein (V) within the PSD. Illustration: Øystein Horgmo, University of Oslo. Similar sections as in (C) at T1-BB demonstrate change in signal intensity of PSD caused by tracer enrichment, before (Pre) (D), and 3 h (E), 6 h (F), 24 h (G) and 48 h (H) after tracer administration. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

parasagittal dura demonstrates that trans-arachnoid transport does occur (Ringstad and Eide, 2020). Previous reports of the parasagittal dura along the middle one-third of the superior sagittal sinus demonstrate presence of numerous intradural arachnoid granulations closely associated with a network of channels from 0.02 to 2.0 mm in diameter (Han et al., 2007; Mack et al., 2009). These channels merge into lateral lacunae from laterally towards the superior sagittal sinus. Recent ultrastructural studies further showed the arachnoid granulations to be in direct contact with subarachnoid CSF and with lymphatic structure expression in pigs (Kutomi and Takeda, 2020). The authors also demonstrated fissures piercing into the dura, large enough for passage of cells, and being covered with endothelial cells and expressing lymphatic structures. Of note is the species differences in dura anatomy (Kinaci et al., 2020), which may explain the differences between observations in rodents and humans.

While MRI in one study was shown useful for demonstrating lymphatic structures (Absinta et al., 2017), the modality is limited by its low resolution. The present MRI has a resolution of 1 mm, which limits the ability to visualize meningeal lymphatic vessels of sub-millimeter cross sectional diameter close to the venous sinus wall (Absinta et al., 2017; Aspelund et al., 2015; Louveau et al., 2015). Thus, the size of parasagittal dura makes it more feasible for semi-quantification of tracer enrichment at MRI, potentially rendering it a useful marker of meningeal lymphatic clearance in man.

At present, there is limited information about the factors determining the function and capacity of meningeal lymphatic vessels, as well as relative importance of ventral/dorsal and cranial/spinal meningeal vessels (Proulx, 2021). Evidence has been given that meningeal lymphatic function becomes impaired with increasing age (Zhou et al., 2020), which may underlie the age-related impairment of molecular clearance from CNS. Another possible factor might be sleep disturbance, but the existing literature has addressed different aspects of sleep. Comparing anesthetized and awake mice showed most efficient meningeal lymphatic function at the skull base during the awake state (Ma et al., 2019). Moreover, the enrichment of CSF tracer in mandibular lymph nodes of anesthetized mice was most pronounced at the time of the day when mice are expected to be most active, suggesting that meningeal lymphatic clearance is under circadian control (Hablitz et al., 2020). The results of these experimental studies may, however, not be directly translated to the present observations, as different aspects of sleep were examined. Meningeal lymphatic function may also be subject to marked species differences.

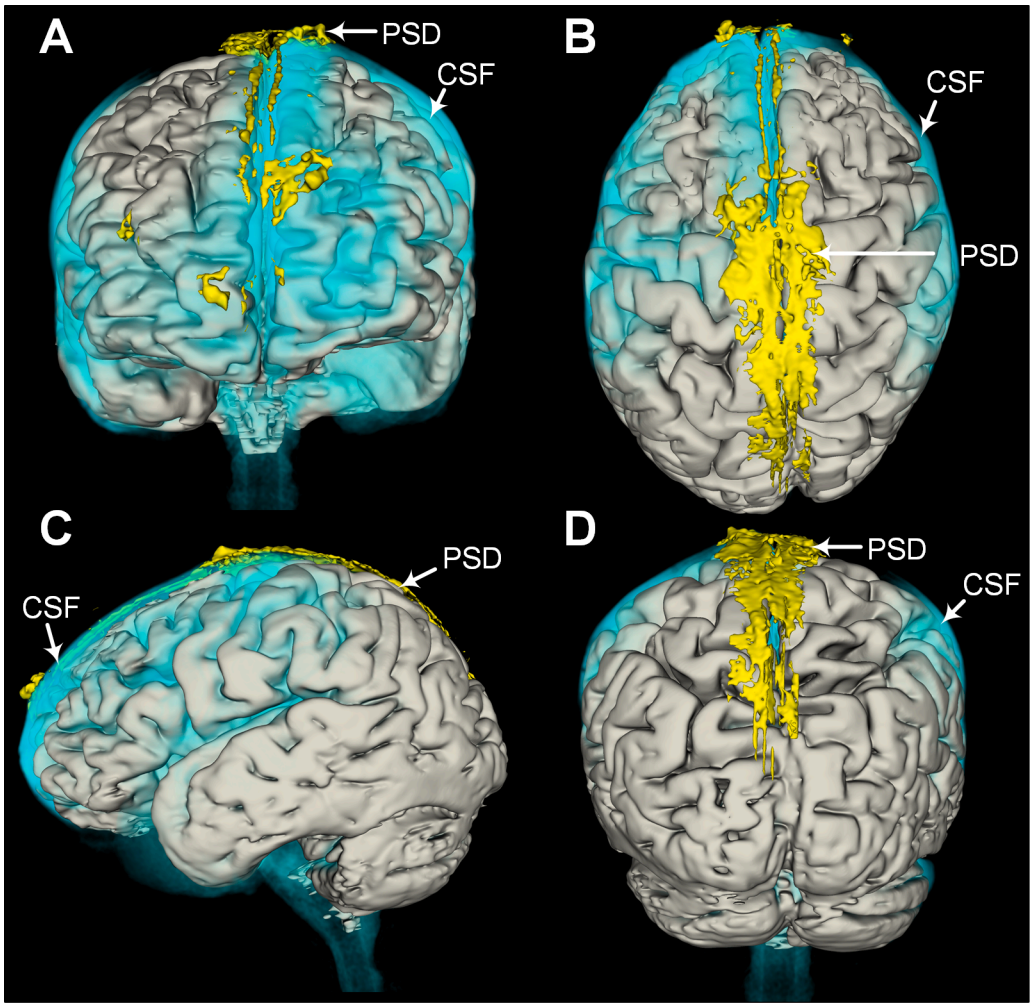


**Fig. 2.** Three-dimensional visualizations of parasagittal dura (PSD) and nearby subarachnoid space enriched by cerebrospinal fluid (CSF) tracer. Visualization of PSD in (A) anterior, (B) upper axial, (C) lateral and (D) posterior views. The PSD (dark yellow) was defined from T2-FLAIR and co-registered with rough segmentation of the brain (grey) and tracer enhancement in subarachnoid CSF (light blue) from T1 GRE at 48 h after intrathecal administration. Images: Tomas Sakinis, MD. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 2**
Correlation between tracer enrichment within the parasagittal dura and nearby subarachnoid CSF over time.

| Parasagittal dura | Subarachnoid CSF | | | |
|---|---|---|---|---|
| | 3 h | 6 h | 24 h | 48 h |
| 3 h | P = 0.75, P = 0.001 | | | |
| 6 h | | P = 0.96, P < 0.001 | | |
| 24 h | | | P = 0.74, P = 0.001 | |
| 48 h | | | | P = 0.29, ns |

Correlations were determined from percentage change in tracer enrichment (normalized T1 signal units) after 3, 6, 24 and 48 h as Pearson correlation coefficients, including the significance level.

Some limitations should be noted. First, MRI with resolution 1 mm does not allow for direct visualization of meningeal lymphatic vessels. It seems reasonable, however, that parasagittal dura communicates directly with nearby lymphatic structures as they do not possess tight junctions. Second, comparing CSF tracer enrichment solely within parasagittal dura during sleep/sleep deprivation may not be full representative for the impact of the sleep-wake state on meningeal lymphatic function. It remains to be determined whether tracer enrichment within parasagittal dura provides a reliable measure of meningeal lymphatic egress capacity.

## 4. Conclusion

The present findings demonstrate that tracer enrichment within parasagittal dura is unaffected by one night's total sleep deprivation, and subsequent sleep. Further studies are needed to determine the impact of sleep-wake state on meningeal lymphatic molecular egress mechanisms.

## 5. Methods and materials

### 5.1. Approvals

The following authorities approved the study: The Institutional Review Board (2015/1868), Regional Ethics Committee (2015/96) and the National Medicines Agency (15/04932-7). Study inclusions was after written and oral informed consent. Participants were registered in Oslo University Hospital Research Registry (ePhorte 2015/1868).

### 5.2. Study protocol

We included patients from a previous study with primary aim to examine the effect of sleep deprivation on glymphatic function in humans (Eide et al., 2021). This present patient cohort incorporates all individuals imaged with MRI-T1 BB sequences, which are particularly suitable for assessing tracer enrichment in parasagittal dura. Data on tracer enrichment in parasagittal dura have not been previously published.

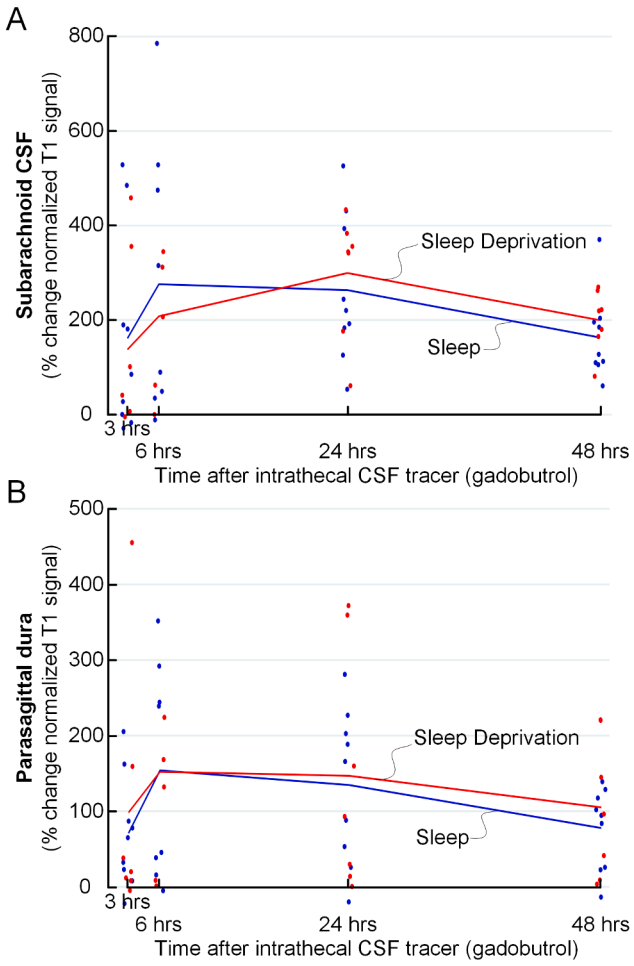


**Fig. 3.** Time course of tracer enhancement within subarachnoid CSF spaces nearby parasagittal dura and within the parasagittal dura. (A) The percentage change in tracer enrichment in subarachnoid CSF space nearby parasagittal dura was comparable between the Sleep Deprivation (red lines) and Sleep (blue lines) cohorts. (B) The percentage change of tracer enrichment within parasagittal dura compared between the Sleep Deprivation (red lines) and Sleep (blue lines) cohorts. There were no differences between groups at any time points, demonstrating that sleep deprivation did not affect molecular egress from parasagittal dura. The trend plots are presented with mean percentage change in normalized T1 signal, and the individual patient observations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The study protocol was to perform MRI scanning with standardized T1-weighted sequences before and on regular time points after intrathecal administration of the MRI contrast agent gadobutrol in two groups of patients: One group underwent total sleep deprivation from Day 1 to 2 whereas another group was allowed unrestricted sleep from Day 1 to Day 2. The total sleep deprivation through 24 h in the Sleep Deprivation group was the intervention. All patients were allowed unrestricted sleep from Day 2 to 3. The MRI contrast agent serves as CSF tracer, which enriches the subarachnoid CSF space and CNS extravascular compartment after administration to the subarachnoid CSF space. We compared tracer enrichments in parasagittal dura and nearby subarachnoid CSF space between the Sleep Deprivation and Sleep groups. The participants who underwent sleep deprivation were observed within the department of neurosurgery by the nursing staff, and a close relative stayed with the participant throughout the night to help he or she stay awake to control that the participants were awake from Day 1 to Day 2.

### 5.3. Patients

We included consecutive patients referred to the Department of neurosurgery, Oslo University Hospital – Rikshospitalet, Oslo, Norway, for tentative CSF disorders, but with final conclusion of no treatable condition after clinical work-up (Table 1). The following exclusion criteria were applied: A history of hypersensitivity reactions to contrast media agents, history of severe allergy reactions in general, or evidence of renal dysfunction (i.e. normal glomerular filtration rate, GFR). Moreover, individuals with age <18 or >80 years, pregnant or breast-feeding women were not included.

Intrathecal gadobutrol administration is performed off-label. Therefore, this study included patients, not healthy controls.

### 5.4. MRI protocol and image analysis

The scans were performed in a 3 Tesla MRI unit (Philips Ingenia®) with a 32-channel head coil.

1. Specifications for whole-brain 3D T2-Fluid Attenuated Inversion Recovery (FLAIR): Repetition time (TR)/echo time (TE)/inversion time (TI) = 4800/311/1650 ms, echo train length = 167, flip angle = 90 degrees, 2 averages, 1 × 1 × 1 mm voxel size (isotropic), and acquisition time 5 min and 41 s.
2. Specifications for whole-brain 3D T1-Black-blood (BB): TR/TE = 700/35 ms, echo train length = 55, flip angle = 80 degrees, 2 averages, 1 × 1 × 1 mm voxel size (isotropic), and acquisition time 4 min and 54 s.
3. Specifications for whole-brain 3D T1-gradient echo (GRE): TR = shortest (typically 5.1 ms); echo time = shortest (typically 2.3 ms); echo train length = 232; flip angle = 8 degrees, 1 average, 1 × 1 × 1 mm voxel size, and acquisition time 6 min and 29 s.

The T1-weighted scans were repeated after 3 and 6 h (Day 1), 24 h (Day 2) and 48 h (Day 3) Patients were kept in the supine position until the 6-hour scan, and were thereafter allowed free movement.

The sagittal T2-fluid FLAIR and T1-BB scans from all time points were reformatted into coronal and axial 1 mm slices perpendicular and parallel to a plane defined by a line between the anterior and posterior commissures (AC-PC plane). Identically positioned coronal slices were used for identification of the parasagittal dura; tissue characteristics

**Table 3**
Tracer enrichment within the parasagittal dura and nearby subarachnoid CSF presented as normalized T1 signal units over time.

| | Pre | | | | 3 h | | | | 6 h | | | | ~24 h | | | | ~48 h | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | Mean | ± | SE | p | Mean | ± | SE | p | Mean | ± | SE | p | Mean | ± | SE | p | Mean | ± | SE | p |
| *Subarachnoid CSF* | | | | | | | | | | | | | | | | | | | | |
| Sleep | 1.46 | ± | 0.07 | 0.001 | 3.73 | ± | 0.74 | ns | 5.38 | ± | 0.78 | ns | 5.20 | ± | 0.74 | ns | 3.83 | ± | 0.74 | ns |
| Sleep Deprivation | 1.13 | ± | 0.08 | | 2.77 | ± | 0.84 | | 3.25 | ± | 0.99 | | 4.52 | ± | 0.84 | | 3.45 | ± | 0.84 | |
| *Parasagittal dura* | | | | | | | | | | | | | | | | | | | | |
| Sleep | 1.52 | ± | 0.05 | <0.001 | 2.59 | ± | 0.48 | ns | 3.78 | ± | 0.50 | ns | 3.49 | ± | 0.48 | ns | 2.68 | ± | 0.48 | ns |
| Sleep Deprivation | 1.20 | ± | 0.06 | | 2.27 | ± | 0.54 | | 2.46 | ± | 0.64 | | 3.01 | ± | 0.54 | | 2.48 | ± | 0.54 | |

Data presented as mean ± standard deviation. P: p-value (mixed model analysis); ns: non-significant.

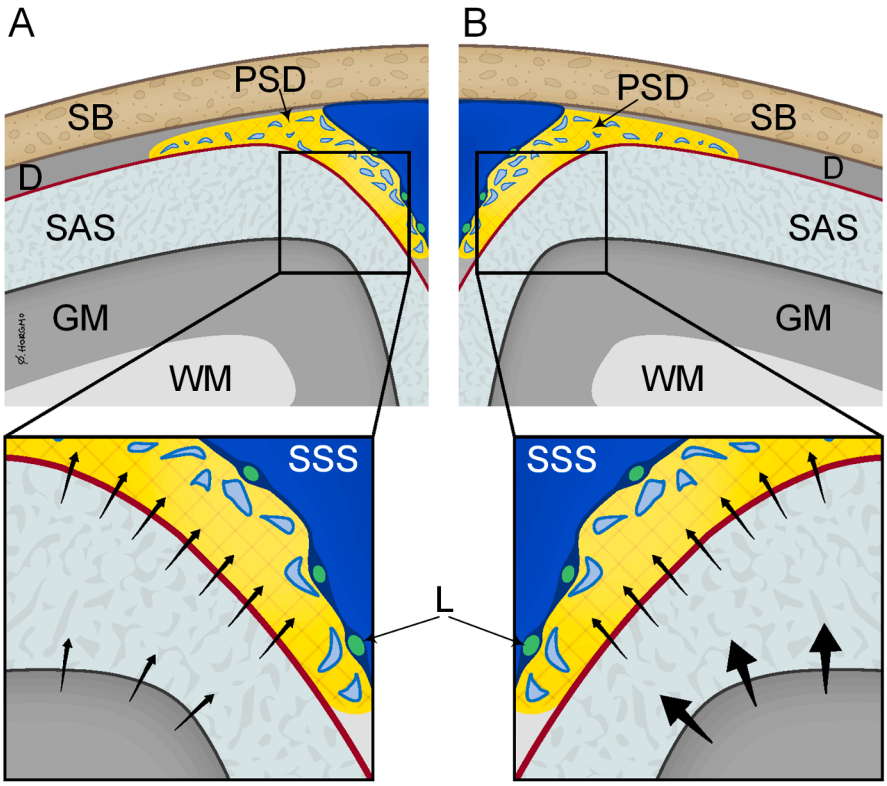


**Fig. 4.** Cartoon illustrating molecular transport during sleep deprivation and sleep in man. In (A) is shown the events occurring during sleep deprivation and in (B) during sleep. The cerebrospinal fluid (CSF) of the subarachnoid space (SAS) is in direct contact with the parasagittal dura (PSD, colored yellow), consisting of a dense carpet of arachnoid granulations and clefts, creating a network of channels within the dura (D), lining the skull bone (SB). Notably, these arachnoid granulations differ from the conventional arachnoid granulations entering into the dural sinus, and previously being thought as a direct passage route for CSF into the venous circulation. The PSD is most prominent at the mid one-third part of the superior sagittal sinus (SSS). Lymphatic vessels (L, green) reside at the SSS wall as tubular structures not visualized on MRI with 1 mm image resolution. The time-dependent enrichment of tracer within PSD and nearby CSF provides evidence for direct molecular passage from subarachnoid CSF to PSD. We suggest that the PSD serves as a direct entrance route to lymphatic pathways. Peak tracer enhancement in PSD also coincides in time with peak tracer enhancement in peripheral lymph nodes (Eide et al., 2018). We have recently provided evidence that brain molecular (glymphatic) clearance depends on sleep, with significantly increased tracer levels in brain tissue after sleep deprivation (Eide et al., 2021). The present data showed no effect of sleep deprivation on molecular egress from CSF to PSD, shown as no change in tracer enrichment in neither the PSD nor the nearby subarachnoid CSF following sleep deprivation. D: dura; GM: grey matter; SAS: subarachnoid space; WM: white matter. Illustrations: Øystein Horgmo, University of Oslo. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

were unsuppressed (intermediate or high signal) at both T2-FLAIR and T1-BB. While T2-FLAIR suppresses free (unbound) CSF within the ventricular compartment and subarachnoid space (Bakshi et al., 2001), T1-BB are tuned to darken the contents of blood vessels, even when containing a contrast agent (Mandell et al., 2017). Accordingly, in e.g. arteries and veins with moving protons, the T1-BB signal is nulled. At the high brain convexities, there is little motion within the CSF, and the T1-BB signal is not black (nulled) as in the blood vessels, but reveals a more intermediate CSF-signal.

Regions of interest (ROIs) were placed in identical locations within parasagittal dura and nearby subarachnoid CSF at all time points at T1-BB images, ensuring that ROIs were well inside the outer perimeter of the parasagittal dura for robust measurement of T1 signal change as sign of tracer enrichment. With regard to nearby CSF, a ROI was placed within adjacent subarachnoid space to assess T1 signal change in CSF. Enrichment of CSF tracer was measured as percentage change in T1-BB signal units. To correct for shifts in baseline of image greyscale between time points, we normalized the signal units for each image set against a reference level defined by a ROI placed within the vitreous body of the ocular bulb, as previously described (Ringstad and Eide, 2020). Moreover, we used the 3D T1 GRE scans for segmentation and co-registration to create 3D representations (using 3D Slicer version 4 and SPM 12, both open source software) and present the relations between the brain surface and CSF tracer enhancement. T1 GRE scans are less suitable for precise ROI-placement in parasagittal dura as the border against the adjacent CSF compartment becomes more or less effaced at late scans due to tracer enrichment, while in T1-BB images these borders remain visibly intact.

### 5.5. MRI contrast agent serving as CSF tracer

The intact blood-brain-barrier (BBB) prevents the contrast agent (gadubutrol; Gadovist, Bayer, GE) used as CSF tracer from leaking into blood vessels within the CNS Furthermore, the tracer is highly hydrophilic with a molecular weight of 604 Da and hydraulic diameter of less than 2 nm. The resulting increased T1 signal thus represents molecular movement in the perivascular and interstitial spaces (i.e. extra-vascular spaces). We therefore consider gadobutrol well suited to serve as surrogate marker for assessing extra-vascular excretion pathways of other water-soluble metabolites, such as some amyloid-β isoforms and tau. Intrathecal administration of gadobutrol is off-label use.

### 5.6. Statistics

SPSS software version 25 (IBM Corporation, Armonk, NY) was applied for statistical analyses, using linear mixed models with a random intercept to determine ddifferences between continuous data, and the Pearson correlation coefficient for determining correlations. Statistical significance was accepted at the 0.05 level (two-tailed).

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## CRediT authorship contribution statement

**Per Kristian Eide:** Conceptualization, Investigation, Formal analysis, Formal analysis, Visualization, Supervision, Project administration, Writing - original draft, Writing - review & editing. **Geir Ringstad:** Conceptualization, Investigation, Formal analysis, Formal analysis,

Visualization, Supervision, Project administration, Writing - original draft, Writing - review & editing.

## Declaration of Competing Interest

Geir Ringstad has been speaker for Bayer AG, Berlin, Germany, The authors declare no other conflict of interests.

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