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Original Article

ORIGINAL ARTICLE

Sleep deprivation and cerebrospinal fluid biomarkers for Alzheimer's disease

Martin Olsson[1,2,3,4,*], Johan Ärlig[1,2,3], Jan Hedner[3,4], Kaj Blennow[1,2] and Henrik Zetterberg[1,2,5,6]

[1]Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden, [2]Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, University of Gothenburg, Sahlgrenska University Hospital, Mölndal, Sweden, [3]Department of Internal medicine, Center for Sleep and Vigilance Disorders, University of Gothenburg, Sahlgrenska University Hospital, Gothenburg, Sweden, [4]Sleep Laboratory, Pulmonary Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden, [5]Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, UK and [6]UK Dementia Research Institute, London, UK

*Corresponding author. Martin Olsson, Neurochemistry, House V, Sahlgrenska University Hospital, SE-431 80, Mölndal, Sweden. Email: martin.v.olsson@vgregion.se.

Abstract

Study Objectives: To investigate the cumulative effect of five consecutive nights of partial sleep deprivation (PSD) on a panel of cerebrospinal fluid (CSF) biomarkers in healthy adults.

Methods: A randomized, cross-over study conducted at the University of Gothenburg. The participants (N = 13) were healthy adults (20–40 years of age) with a normal sleeping pattern. The participants underwent a baseline sleep period consisting of five nights with 8 hr spent in bed. A subsequent period with PSD consisted of five nights of maximum 4 hr of sleep per night. Four participants were also subjected to a prolonged period of PSD consisting of eight nights with 4 hr of sleep per night. Sleep was monitored by means of observation, actigraphy, and continuous polysomnographic recordings. CSF samples were collected by routine lumbar puncture after each period. CSF biomarkers included the 38, 40, and 42 amino acid–long Aβ isoforms, total-τ, phospho-τ, orexin, monoamine metabolites (3-methoxy-4-hydroxyphenylglycol, homovanillinic acid, and 5-hydroxyindoleacetic acid), neuron-derived biomarkers (neurofilament light, neuron-specific enolase, and fatty acid–binding protein), and astro- and microglia-derived biomarkers (glial fibrillary acidic protein, S-100B, and YKL-40).

Results: PSD was associated with a 27 per cent increase in CSF orexin concentrations (p = 0.001). No PSD-related changes in CSF biomarkers for amyloid build-up in the brain, Alzheimer's disease (AD)-type neurodegeneration, or astroglial activation were observed. PSD led to a shortening of time spent in all sleep stages except slow-wave sleep (SWS).

Conclusion: Five to eight consecutive nights of PSD, with preserved SWS, increased CSF orexin but had no effect on CSF biomarkers for amyloid deposition, neuronal injury, and astroglial activation.

Statement of Significance

Recent studies suggest an association between sleep loss and reduced clearance from the brain of the Alzheimer's disease (AD) associated peptide amyloid β. However, research investigating the effect of longer partial sleep deprivation (PSD) on cerebrospinal fluid (CSF) biomarkers for AD pathology is sparse. There is also a lack of knowledge on how other common CSF biomarkers respond to PSD.

Key words: Alzheimer's disease; amyloid β; neuron-specific enolase; orexin; monoamine; S100 calcium-binding protein B; sleep deprivation; sleep loss; sleep; cerebrospinal fluid

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Introduction

Sleep may have profound effects on both the production and clearance of a number of central nervous system (CNS)–derived proteins and metabolites of relevance to Alzheimer's disease (AD) and other neurodegenerative diseases. Sleep influences neuronal activity that in turn affects the release of, e.g., amyloid β (Aβ) and τ from neurons [1]. Furthermore, the brain depends on the glymphatic system for clearance of proteins and metabolites from the brain interstitial fluid (ISF) to the blood and the cerebrospinal fluid (CSF) [2, 3]. Animal studies have suggested an association between sleep and increased glymphatic efflux of proteins, including Aβ, and metabolites from the brain parenchyma. However, this has not been well investigated in humans yet [3].

Aβ, or more specifically the Aβ42 isoform, is the key component of senile plaques associated with AD [4]. A recent study on healthy volunteers showed that one night of total sleep deprivation (TSD) interferes with a physiological morning decrease in Aβ42 [5]. Other data suggest a relationship between loss of sleep and/or sleep fragmentation and a risk of developing AD [6, 7].

Orexin is a neuropeptide that plays a crucial role as a switch between wakefulness and sleep [8]. There is an interesting association among sleep debt, orexin secretion, and AD. Orexin gene knock out mice have been shown to have less AD pathology [9]. This could possibly mean that orexin and sleep debt may be an upstream driver of AD.

In this study, we examined the cumulative effect of five or eight consecutive nights of partial sleep deprivation (PSD) in healthy adults on CSF concentrations of several biomarkers reflecting key aspects of AD neuropathology, including amyloidogenic processing of Aβ precursor protein (APP; Aβ38 and -40), amyloid build-up in the brain (Aβ42), AD-type neurodegeneration (total-τ [T-τ] and phospho-τ [P-τ]), other types of neuronal injury (neurofilament light [NF-L], fatty acid–binding protein [FABP], and neuron-specific enolase [NSE]), and astroglial activation (glial fibrillary acidic protein [GFAP], S-100B and YKL-40). We also measured monoamine metabolite and orexin concentrations in CSF. We hypothesized that prolonged wakefulness in PSD would reduce the physiological clearance of CSF biomarkers associated with AD. Furthermore, we hypothesized that the effect on CSF Aβ42 levels, compared with control, would be more pronounced than previously witnessed after one night of TSD [5]. Finally, we hypothesized that orexin would increase after PSD as a result of sleep debt and that other markers of neuronal injury and/or astroglial activation would change in response to PSD.

Methods

Participants

Sixteen healthy participants were recruited by advertisement. Inclusion criteria were age of 20 to 40 years and a typical sleep pattern, defined as self-reported normal bedtime before midnight, regular morning awakening between 06.00 and 09.00 am, and a habitual sleep duration of 6.5 to 8.5 hr. Exclusion criteria included body mass index (BMI) > 30 kg/m^2, continuous use of medication or relevant chronic diseases, history of a sleep disorder (e.g. chronic insomnia, daytime sleepiness, or narcolepsy), Epworth Sleepiness Scale (ESS) score >10, and a self-reported average sleep latency ≥20 min. The use of caffeine, nicotine, or any vigilance-modulating substances was prohibited during the period of the experiment.

Study design

The participants were subjected to a period of PSD, consisting of five consecutive nights with a maximum of 4 hr of sleep per night. During the PSD period, participants arrived at the sleep laboratory at 10:00 pm each night. The participants were constantly monitored and bedtime was set to between 03:00 and 04:00 am. Wake up time was set exactly 4 hr after lights out and participants were woken by laboratory personnel. The protocol was established in accordance with that of a previously published study following slight modifications [10]. While at the sleep laboratory, participants were limited to one standardized meal consisting of less than 500 kcal per night, during the PSD period. Furthermore, the participants underwent a period of controlled sleep (CS) consisting of five consecutive nights of 8 hr spent in bed each. Bed time was set to between 10:00 and 11:00 pm. The CS and PSD periods were randomized in order and separated by at least 4 weeks of normal sleep without interference. Half of the study group started with the PSD period prior to the period of CS, whereas the other half had the opposite arrangement. The study flow chart is shown in Figure 1. In an ad hoc experiment, four participants, who had completed the main PSD protocol, were subjected to a prolonged PSD consisting of 8 days of restricted sleep. Apart from the number of days, the protocol was identical with the shorter PSD protocol.

This study was approved by the Ethical Committee for Medical Research at the University of Gothenburg and was conducted in accordance with the Helsinki declaration. Oral and written informed consent was obtained from all study participants prior to enrollment.

Sleep surveillance

Polysomnography (PSG) was used to assess sleep duration and sleep stages throughout the PSD protocol and during the first and last night of the CS period. The first night was used for habituation. The PSG recording montage included electroencephalography (EEG), electrooculography (EOG), electromyography (EMG), and electrocardiography (ECG). Electrode placement for the EEG included the F4, C3, C4, A1, A2, and O1 locations. EOG electrodes were placed at standard paraocular positions. EMG electrodes were placed above and below the chin. One ECG trace was recorded by using bilateral clavicular electrodes. PSG recordings were scored according to American Association of Sleep Medicine (AASM) guidelines [11] by an external-registered PSG technologist, using a commercially available software (Remlogic) and blinded to the study code.

ActiGraph GT3X+ devices were worn on the nondominant wrist, throughout the experiment. Data from the devices were used, in parallel, to assess sleep duration during the CS period as well as protocol adherence throughout the study protocol. Participants were encouraged, on a daily basis, to report events that could affect protocol adherence. Total sleep time (TST) ≤ 420 min per night during the CS period was considered as protocol nonadherence and led to exclusion from the study. Actigraphy data were reviewed with the ActiLife software and analyzed with the Sadeh algorithm [12, 13].

CSF sampling and analysis

CSF samples were collected by lumbar puncture at the L3/L4 or L4/L5 interspace with a 22 g × 90 mm Sprotte needle, by an experienced neurologist. This type of needle is known to minimize the risk of postdural puncture headache [14]. Sampling was performed at 08:00 to 09:00 am on the first morning after completion of each period (CS, PSD, and prolonged PSD). Samples collected after the CS period acted as control. Ten to twelve milliliters of CSF were collected in polypropylene tubes, centrifuged at 1300 g for 10 min, aliquoted, and stored in 0.5 mL aliquots at –80°C pending analysis within 1 hr after sampling.

CSF Aβ38, Aβ40, and Aβ42 concentrations were measured using both MSD Abeta Triplex (Meso Scale Discovery, Rockville, Maryland) and EUROIMMUN (Euroimmun AG, Lübeck, Germany) assays. CSF T-τ, P-τ, and Aβ42 concentrations were measured using INNOTEST sandwich enzyme–linked immunosorbent assays (ELISAs, Fujirebio, Ghent, Belgium). CSF T-τ was also measured using the EUROIMMUN kit (Euroimmun AG, Lübeck, Germany). CSF NF-L concentration was measured using the NF-Light ELISA (UmanDiagnostics, Umeå, Sweden). CSF concentrations of NSE and S-100B were measured using the Modular system (Cobas E601) and NSE and S-100B reagent

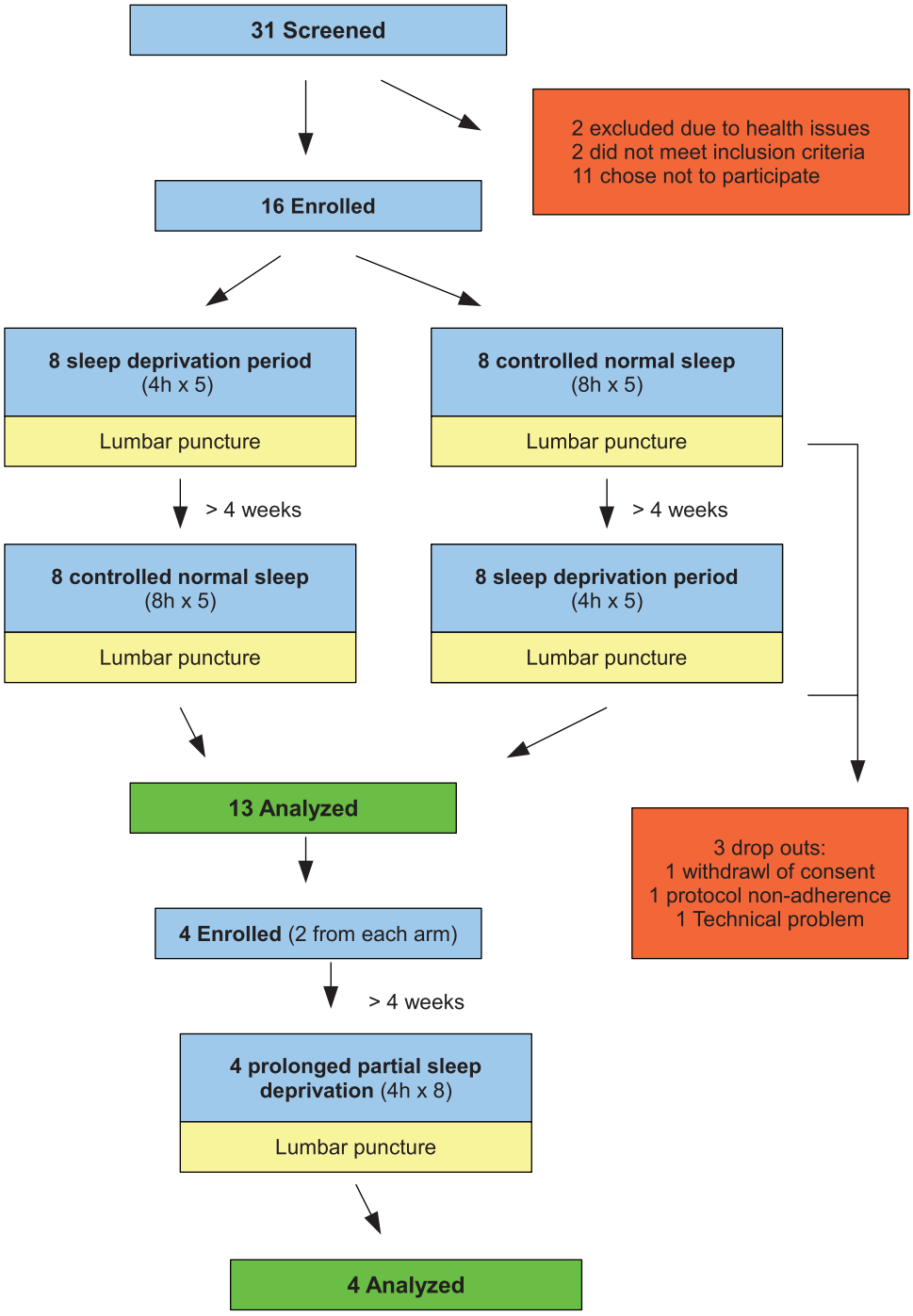

Figure 1. Study flowchart.

kits (Roche Diagnostics, Basel, Switzerland). CSF FABP concentration was measured using an MSD electrochemiluminescent assay (Meso Scale Discovery, Rockville, Maryland). CSF YKL-40 (also called chitinase 3-like 1) concentration was measured using the Human Chitinase 3-like 1 Quantikine ELISA Kit (R&D Systems, Inc., Minneapolis, MN). CSF GFAP concentration was measured using an inhouse ELISA, as described previously [15]. CSF orexin concentration was measured using an in-house radioimmunoassay (RIA), as described previously [16]. CSF concentrations of the dopamine metabolite homovanillinic acid (HVA), the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA), and the norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) were measured using high-performance liquid chromatography (HPLC) with electrochemical detection, as described previously [17]. All measurements were performed in one round of experiments with one batch of reagents and baseline and follow-up samples side by side on the assay plates by board-certified laboratory technicians who were blinded to clinical data.

Statistical methods

All analyses were performed using SPSS (version 23.0, IBM, Chicago, USA). Statistical significance was set to $p < 0.05$. All data are presented as mean and ±*SD*. Normality was assessed by the Shapiro–Wilk test. As much of the data were not normally distributed, within-group comparisons were addressed by paired samples analysis with Wilcoxon signed-rank test. Correlations were examined using the Spearman rank correlation coefficient.

Results

Participant characteristics and drop outs

A total of 13 participants were included in the study ($N = 13$). Three participants were excluded from statistical analysis: one due to nonadherence to protocol (TST ≤ 420 min during a CS night), another one due to technical difficulties with the PSG during PSD, and a third participant due to withdrawn consent. Baseline characteristics and anthropometric data are summarized in Table 1.

Table 1. Anthropometric and baseline characteristics

Variable	Complete study population ($N = 13$)
Anthropometric variable	Mean (*SD*)
Age, y	25 (4.0)
Weight, kg	79.3 (13.6)
Height, cm	184.2 (14.0)
BMI	23.4 (2.4)
Pulse	60 (6)
Systolic BP, mmHg	134 (5)
Diastolic BP, mmHg	81 (6)
ESS	6 (3)
Baseline characteristics	No. (%)
Gender, male	9 (69.2)
Nicotine, smoker	3 (23.1)
Alcohol, >15 standard units	1 (6.2)

BMI = body mass index; BP = blood pressure; ESS = Epworth Sleepiness Scale.

Sleep parameters

Average TST, assessed by PSG, during the last night of the CS period was 7.3 hr. All mean sleep durations for each period and time spent in each sleep stage during the CS and PSD periods are summarized in Table 2.

During the PSD period, PSG was collected and analyzed all five nights (Table 2). There was no difference between the CS and PSD periods in terms of duration of slow-wave sleep (SWS)/non-rapid eye movement (NREM) stage 3, whereas NREM stage 1, NREM stage 2, and rapid eye movement (REM) sleep was significantly (all $p = 0.02$) reduced by 68% (19 min), 64% (131 min), and 46% (52 min), respectively.

During the time spent outside of the sleep lab, four episodes were interpreted by the actigraphy software as sleep. All four episodes were discovered to be from user errors such as taking off the actigraph while swimming and forgetting to put it back on. No episode of "true out of protocol" sleep was registered, though the algorithm is not sensitive for very short periods of sleep.

CSF biomarker results

No significant changes in CSF concentrations reflecting amyloidogenic APP processing, cerebral β-amyloidosis, neuronal injury, or astroglial activation were detected in the samples collected immediately after the PSD period compared with samples from the CS period (Table 3). As expected, CSF orexin concentration increased by 27 per cent, from 643 pg/mL to 818 pg/mL ($p = 0.001$) following sleep deprivation (Table 3). There were no PSD-related changes in the CSF concentrations of any of the monoamine metabolites. In the prolonged PSD arm, orexin increased by 21 per cent from 640 pg/mL to 771 pg/mL. No other relevant changes were seen. Significance was not tested in this ad hoc study due to the small sample size ($N = 4$). No significant correlations were found between biomarkers and sleep spent in non-REM stage 1, 2, 3 or REM sleep either in the PSD or prolonged PSD group.

Discussion

Our study confirmed an increase in CSF orexin concentration after five or eight nights of PSD but did not reveal any PSD-related changes in the concentrations of biomarkers for amyloid deposition, neuronal injury, or astroglial activation (Table 3, Figure 2, A–C). These results speak against any major effect of PSD on the turnover of these proteins within the CNS. An additional way of interpreting the results is that PSD during five or eight nights does not seem to cause acute neuronal damage, at least not in a way that can be detected with the CSF markers for neuronal injury and astroglial activation that we used.

One important limitation of this study is the small study population. Because of this, further stratification of data in relation to sleep stages and their relation to specific biomarkers is not possible. Furthermore, there is support of a diurnal variation of CSF Aβ concentrations [18]. However, in our study, all samples were taken at the same time point. The timing of the CSF collection was chosen to avoid contamination of the results by daytime activities of the study participants. This means that timing in regards to diurnal fluctuation had to be sacrificed. In an ideal experiment, participants would have stayed still in bed, but awake, for approximately 4 hr before lumbar punctures were to be performed. Our rationale for this decision was that

Table 2. Overview of sleep data

Variable	Controlled sleep (*N* = 13)	Mean (*SD*) Partial sleep deprivation (*N* = 13)	*P*
PSG variable, min			
TST	438.3 (27.7)	231.3 (4.4)	0.02*,†
NREM stage 1	28.4 (12.9)	9.0 (5.8)	0.02*,†
NREM stage 2	204.3 (28.1)	73.4 (15.7)	0.02†,*
SWS/NREM stage 3	91.6 (21.5)	86.9 (16.2)	0.347†
REM	114.0 (21.4)	62.0 (8.2)	0.02*,†
Actigraphic variable, min			
TST	482.7 (46.8)	230,00 (56,2)	0.02†,*

PSG = polysomnography; TST = total sleep time; NREM = nonrapid eye movement sleep; SWS = slow-wave sleep; REM = rapid eye movement sleep.
†*p*-Values represent within group (the same participants exposed to two sleep conditions) differences between the last night of polysomnographic recording during controlled normal sleep and the average over five nights of polysomnographic recording during the partial sleep deprivation period.
**p*-Value < 0.05.

our primary objective was to investigate whether there were any PSD-induced cumulative changes in the CSF composition. Hypotheses relating to whether there are PSD-induced changes in the diurnal fluctuation of CSF biomarkers need to be examined using a different study design.

From a technical standpoint, actigraphy is inferior to PSG in several ways. It is less sensitive to short periods of sleep (naps) and it showed to be less reliable as indicated by the large standard deviation seen in Table 2 (TST as measured by actigraphy). It is possible that participants experienced short periods of sleep while outside of the lab setting. This could have possibly decreased our chance of finding significant biomarker changes. However, keeping test participants at the sleep lab throughout the experiment would have its own set of drawbacks.

Contrary to our hypothesis, there were no changes in the CSF concentrations of any of the biomarkers reflecting AD pathology, neuronal cell damage, or astroglial activation after five or eight nights of PSD. This puts some new light on previous theories on how protein clearance from the brain parenchyma into the CSF may be affected by sleep deprivation. Animal and human studies suggest that sleep induces an increase in fluid exchange between the brain ISF and the CSF including an increased clearance of Aβ42 and other CNS-derived proteins and metabolites [3]. One night of TSD has been shown to increase CSF Aβ42 morning levels compared with unrestricted sleep in healthy middle-aged men [5]. Other recent data indicate that increased CSF Aβ in the morning after TSD is a result of a change in production rather than

Table 3. CSF biomarker data

Baseline (CS) vs PSD		Mean (*SD*)		
Variable	Baseline (CS) (*N* = 13)	Partial sleep deprivation (*N* = 13)	*P*	Prolonged PSD (*N* = 4)
CSF value				
Orexin, pg/mL	642.6 (127.0)	818.2 (159.6)	0.001*,†	771.3 (188.0)
Monoamine metabolites				
HVA, nmol/L	174.2 (65.5)	184.2 (63.3)	0.53†	177.8 (33.1)
5-HIAA, nmol/L	79.6 (25.7)	79.2 (18.0)	0.97†	77.3 (18.4)
MHPG, nmol/L	39.8 (6.5)	39.7 (7.1)	0.96†	38.8 (10.0)
Amyloid and associated biomarkers				
T-τ, innotest‡, pg/mL	208.5 (80.5)	215.5 (85.0)	0.44†	188.2 (55.0)
P-τ, innotest‡, pg/mL	38.1 (12.8)	39.4 (12.8)	0.12†	37.3 (8.7)
Aβ42, innotest‡, pg/mL	1063.4 (150.7)	1085.7 (152.9)	0.13†	986.0 (149.7)
Aβ38, MSD§, pg/mL	2551.5 (710.2)	2639.7 (768.6)	0.46†	2370.8 (602.0)
Aβ40, MSD§, pg/mL	7242.4 (1695.0)	7432.4 (1640.6)	0.35†	6594.6 (1410.8)
Aβ42, MSD§, pg/mL	891.2 (240.7)	912.7 (247.1)	0.38†	800.6 (196.0)
Aβ38, Adx‖, pg/mL	2004.3 (512.3)	2048.4 (610.8)	0.43†	1845.7 (467.2)
Aβ40, Adx‖, pg/mL	7044 (2214.6)	7514.6 (2177.3)	0.09†	6452.4 (1870.8)
Aβ42, Adx‖, pg/mL	1042.5 (300.3)	1035.7 (309.9)	0.97†	914.3 (263.8)
T-Tau, Adx‖, pg/mL	206.8 (60.0)	212.9 (73.4)	0.62†	181.8 (34.0)
Neuron-derived biomarkers				
NFL, pg/mL	384.8 (355.2)	325.2 (200.2)	0.46†	440.3 (289.1)
NSE, ng/mL	5.5 (1.6)	5.7 (1.9)	0.36†	5.0 (1.8)
FABP, ng/mL	3.5 (1.4)	3.5 (1.5)	0.50†	3.25 (0.8)
Astrocyte/Microglial-derived biomarkers				
GFAB, pg/mL	187.6 (73.5)	182.8 (70.4)	0.80†	176.1 (79.6)
S100B, pg/mL	0.7 (0.2)	0.7 (0.1)	0.44†	0.7 (0.2)
YKL-40, pg/mL	56474.4 (26285.4)	56718.5 (24812.4)	0.92†	50120.9 (28928.7)

CSF = cerebrospinal fluid; CS = controlled sleep; PSD = partial sleep deprivation; FABP = fatty acids–binding proteins; HVA = homovanillic acid; 5-HIAA = 5-hydroxyindoleacetic acid; MHPG = 3-metoxy-4-hydroxyphenylglycol; Aβ = β-amyloid; T-τ = total-τ; P-τ = phosporylated-τ; NFL = neurofilament light; NSE = neuron-specific enolase; GFAP = glial fibrillary acidic protein; S100-B = calcium-binding protein B; YKL-40 = chitinase-3-like protein.
†*p*-Values represent within-group (the same participants exposed to two sleep conditions) differences for the controlled sleep period samples compared with the partial sleep deprivation samples.
‡Fujirebio Innotest ELISA.
§Aβ peptide panel 6E10 MSD ELISA.
‖Euroimmun Adx ELISA.
**p*-Value < 0.01.

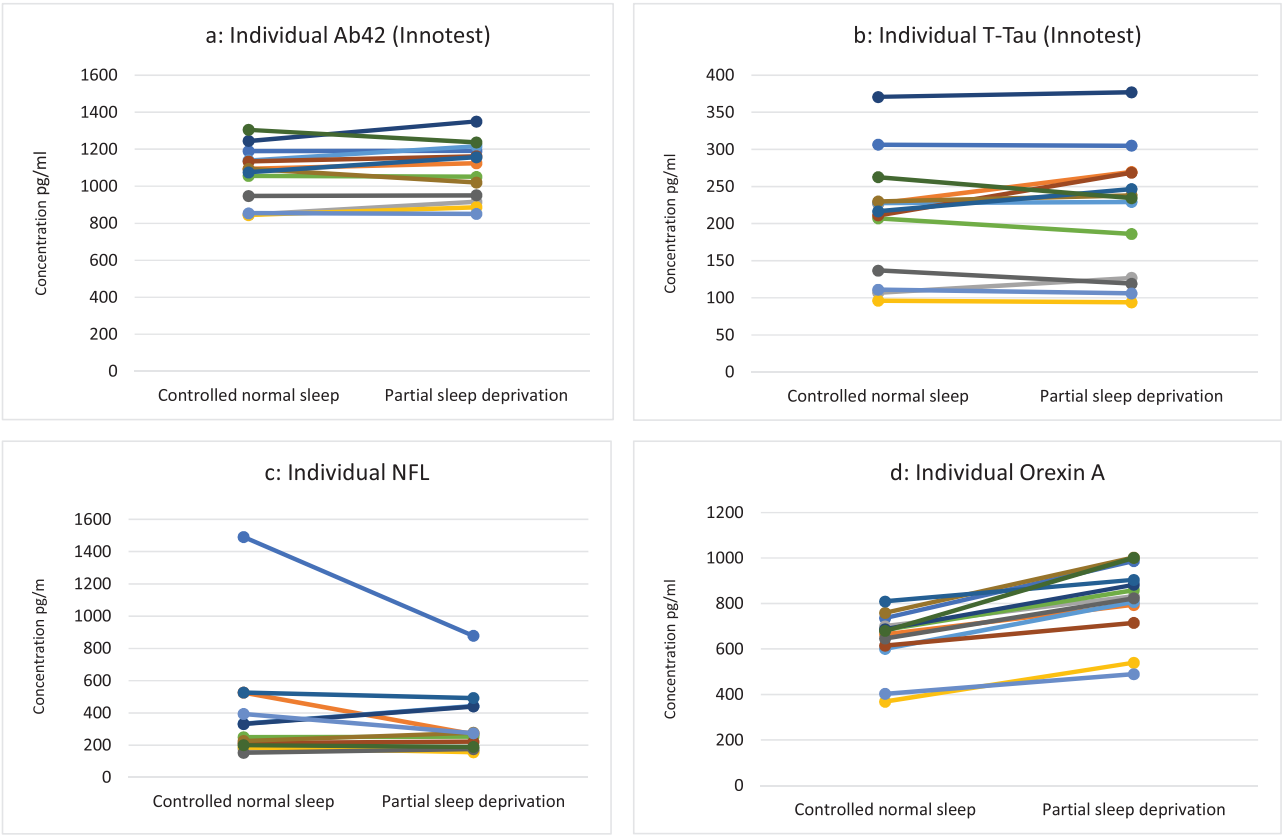

Figure 2. Individual test participant CSF concentration of Aβ42, τ, NFL, and orexin A. Controlled sleep vs. partial sleep deprivation.

clearance [19]. We hypothesized that exposure to five consecutive nights of PSD would disrupt normal CSF dynamics during sleep and result in a similar relative increase of the CSF biomarkers.

A possible explanation for the discrepancies between our findings, our early hypothesis, and previous findings may be an altered sleep structure experienced by sleep-deprived test participants. We used an established PSD protocol since we expected this to more closely reflect sleep disturbances as they occur in the general population. However, with our PSD protocol, because of rebound sleep, there was no decrease in time spent in SWS compared with controlled normal sleep. TSD on the other hand completely eliminates SWS.

A recent study showed increased CSF Aβ42 levels after healthy adults underwent a protocol of normal sleep duration but with automated SWS disruption [20]. Our data further support the observation that SWS seems particularly important for clearance and/or decrease in production of, at least, the proteins we measured. What physiological characteristics of SWS that is responsible for this effect is not certain but the electrophysiological synchronization that occurs in this state of sleep could potentially affect both neuronal activity and clearance. Maybe synchronization is of key importance for bulk efflux and influx of fluid to and from the ISF.

Sleep stage-dependent CSF protein dynamics, as suggested by our data, raises questions about when it is appropriate to use TSD protocols in neurochemical research. TSD and PSD protocols both appear to have their place but data obviously need to be interpreted with caution.

There is a well-known association between AD and disturbed sleep [21], and AD is commonly linked to reduced REM sleep and SWS [22]. There is also an increased REM sleep onset latency in AD [23]. Sleep deficiency has also been hypothesized to be a driving force behind Aβ deposition in AD, either by decreased clearance [24] or by increased Aβ production because of extended wakefulness [25]. Our data do not rule out a possible association between loss of SWS and increased CSF Aβ concentration, but it demonstrates that REM sleep may be less important for Aβ clearance, as our test participants' Aβ levels were not affected by PSD even though REM duration was distinctly decreased during PSD.

Research suggests that REM sleep deprivation, specifically, increases orexin concentration. This seems to be true both in induced and acute SD [26], as well as in chronic sleep deprivation associated with AD. In this study, orexin followed this expected pattern, with increased CSF concentration after PSD. As previously mentioned, there are data to suggest that orexin may play a role in the development of AD [9, 27]. Although we did not observe an increase in AD-associated biomarkers, there may still be an effect of orexin since we only investigated CSF and not all possible sites of protein build-up, such as ISF or the intracellular space. Orexin seems to have a proportional promoting effect on REM and NREM sleep [28], indicating that orexin is not responsible for the disproportional decrease in REM sleep seen during PSD in our experiment. Interestingly, we did not see a further increase in CSF orexin concentrations after 8 days of PSD, compared with 5 days of PSD. This suggests a possible ceiling effect on orexin production. Further research on human orexin dynamics in regards to sleep and sleep deprivation would be valuable.

Our study could not identify an increased concentration of AD-associated biomarkers after five to eight nights of PSD with preserved SWS. Protein clearance and/or production dynamics

appears to be different in prolonged PSD compared with TSD. The explanation for this difference may reside in the maintained residual SWS in PSD.

Authors' Contributions

Zetterberg and Olsson had full access to the data in the study and take full responsibility of the accuracy of data analysis and the integrity of the data. Study concept and design: Olsson, Ärlig, Hedner, Blennow, and Zetterberg. Obtained funding: Hedner, Blennow, and Zetterberg. Study supervision: Hedner, Blennow, and Zetterberg. Data acquisition, analysis and/or interpretation: Ärlig, Olsson, Hedner, Blennow, and Zetterberg. Statistical analysis: Ärlig, Olsson, and Zetterberg. Drafting of the manuscript: Ärlig and Olsson. Manuscript revision for critical intellectual content: All authors.

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Notes

Conflict of interest statement. K.B. and H.Z. are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg.

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