Sleep Deprivation and CSF Biomarkers for Alzheimer Disease

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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, Zetterberg *Obtained funding:* Hedner, Blennow, Zetterberg. *Study supervision:* Hedner, Blennow, Zetterberg. *Data acquisition, analysis and/or interpretation:* Ärlig, Olsson, Hedner, Blennow, Zetterberg. *Statistical analysis:* Ärlig, Olsson, Zetterberg. *Drafting of the manuscript:* Ärlig, Olsson. *Manuscript revision for critical intellectual content:* All authors

Abstract

Study Objective To investigate the cumulative effect of 5 consecutive nights of partial sleep deprivation 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 5 nights with 8h spent in bed. A subsequent period with partial sleep deprivation (PSD) consisted of 5 nights of maximum 4h of sleep per night. Four participants were also subjected to a prolonged period of PSD consisting of 8 nights with 4h 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-tau, phospho-tau, orexin, monoamine metabolites (MHPG, HVA and 5-HIAA), neuron-derived biomarkers (NF-L, NSE, FABP) and astro- and microglia-derived biomarkers (GFAP, S-100B, YKL-40).

Results PSD was associated with a 27% increase in CSF orexin concentrations (P= 0.001). No PSD-related changes in CSF biomarkers for amyloid build-up in the brain, Alzheimer 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 5-8 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.

Keywords Alzheimer disease, amyloid beta, neuron-specific enolase, orexin, mono amine, S100 calcium binding protein B, sleep deprivation, sleep loss, sleep, cerebrospinal fluid.

Statement of Significance

Recent studies suggest an association between sleep loss and reduced clearance from the brain of the Alzheimer disease (AD) associated peptide amyloid β (Aβ). However, research investigating the effect of longer 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.

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 disease (AD) and other neurodegenerative diseases. Sleep influences neuronal activity that in turn affects the release of, *e.g.*, amyloid β (Aβ) and tau from neurons (1). Further, 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 Alzheimer disease (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 suggests 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 between 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 5 or 8 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-tau [T-tau] and phospho-tau [P-tau]), 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 1 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 and materials

Participants

Sixteen healthy subjects 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 hours. Exclusion criteria included Body Mass Index (BMI) > 30 kg/m², 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 minutes. 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 four hours of sleep per night. During the PSD period subjects arrived at the sleep laboratory at 10 pm each night. The participants were constantly monitored and bedtime was set to between 3 and 4 am. Wake up time was set exactly four hours 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 eight hours spent in bed each. Bed time was set to between 10 and 11 pm. The CS and PSD periods were randomized in order and separated by at least four weeks of normal sleep without interference. Half of the study group started with the PSD period prior to the period of CS whilst the other half had the opposite arrangement. The study flow chart is shown in **Figure 1**. In an *ad hoc* experiment, 4 subjects, 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 were 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 was 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 ≤420 min per night during the CS period was considered as protocol non-adherence and led to exclusion from the study. Actigraphy data was 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 22gx90mm Sprotte™ needle, by an experienced neurologist. This type of needle is known to minimize the risk of post dural puncture headache (14). Sampling was performed at 8 to 9 am on the first morning after completion of each period (CS, PSD and prolonged PSD). Samples collected after the CS period acted as control. 10-12 mL of CSF was collected in polypropylene tubes, centrifuged at 1300g for 10 min, aliquoted and stored in 0.5 mL aliquots at -80°C pending analysis within 1h 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-tau, P-tau and Aβ42 concentrations were measured using INNOTEST sandwich enzyme-linked immunosorbent assays (ELISAs, Fujirebio, Ghent, Belgium). CSF T-tau 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 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 previously described (15). CSF orexin concentration was measured using an in-house radioimmunoassay (RIA), as previously described (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 previously described (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 is 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

Subject characteristics and drop outs

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

Sleep parameters

Average total sleep time (TST), assessed by PSG, during the last night of the CS period was 7.3 hours. 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 SWS/NREM stage 3 while NREM stage 1, NREM stage 2 and 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%, 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% 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 5 or 8 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 2a-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 5 or 8 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 subjects would have stayed still in bed, but awake, for approximately 4 hours before lumbar punctures were to be performed. Our rationale for this decision was that our primary objective was to investigate if 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 (total sleep time 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 subjects 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 5 or 8 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 total sleep deprivation has been shown to increase CSF Aβ42 morning levels compared with unrestricted sleep in healthy middle-aged men (5). Other recent data indicates that increased CSF Aβ in the morning after total sleep deprivation is a result of a change in production rather than clearance (19). We hypothesized that exposure to 5 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 subjects. 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 slow wave sleep (SWS) compared with controlled normal sleep. Total sleep deprivation 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 supports 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 synchronisation 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 total sleep deprivation protocols in neurochemical research. TSD and PSD protocols both appear to have their place but data obviously needs to be interpreted with caution.

There is a well-known association between and 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 does 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 subjects' 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 partial sleep deprivation. As previously mentioned, there is 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, as compared to 5 days of PSD. This suggest 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 5-8 nights of partial sleep deprivation with preserved slow wave sleep. Protein clearance and/or production dynamics appears to be different in prolonged partial sleep deprivation as compared to total sleep deprivation. The explanation for this difference may reside in the maintained residual SWS in PSD.

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Role of Sponsor

The funding sources had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; and preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Conflict of interest disclosure

KB and HZ 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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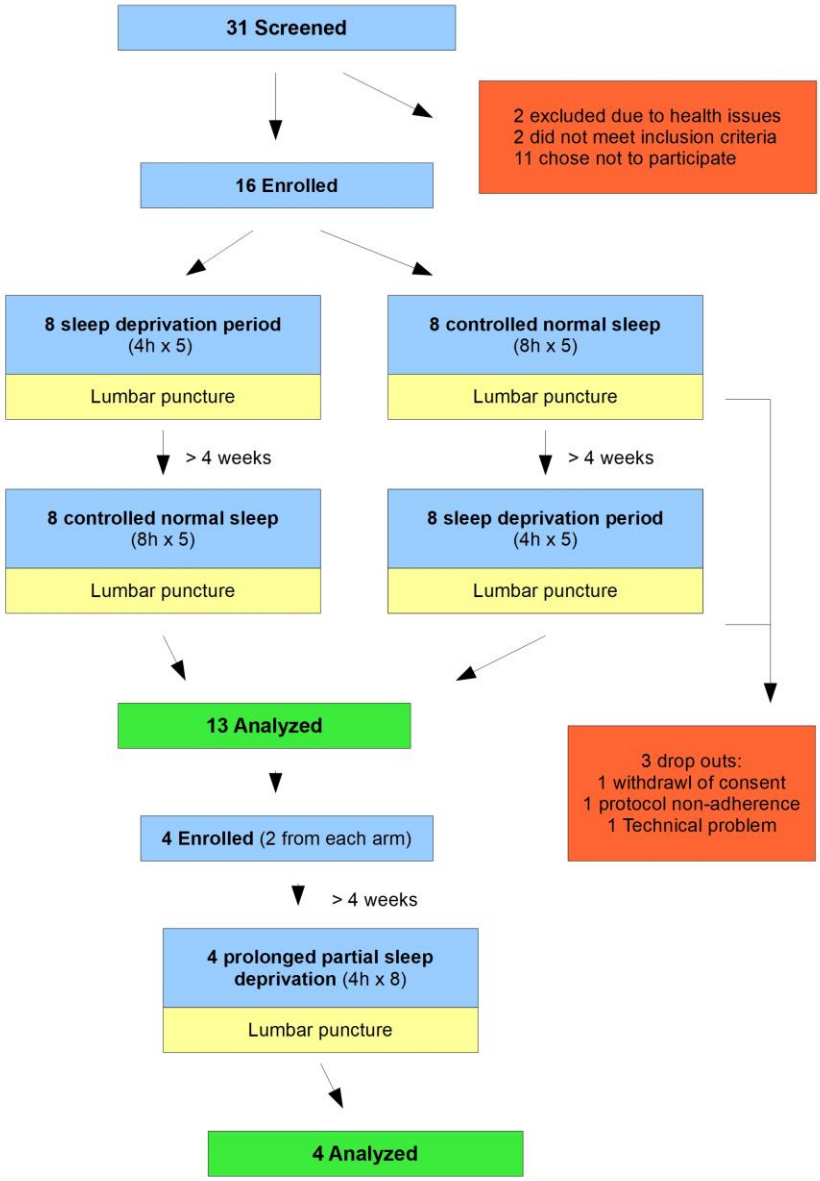


Figure 1. Study flowchart

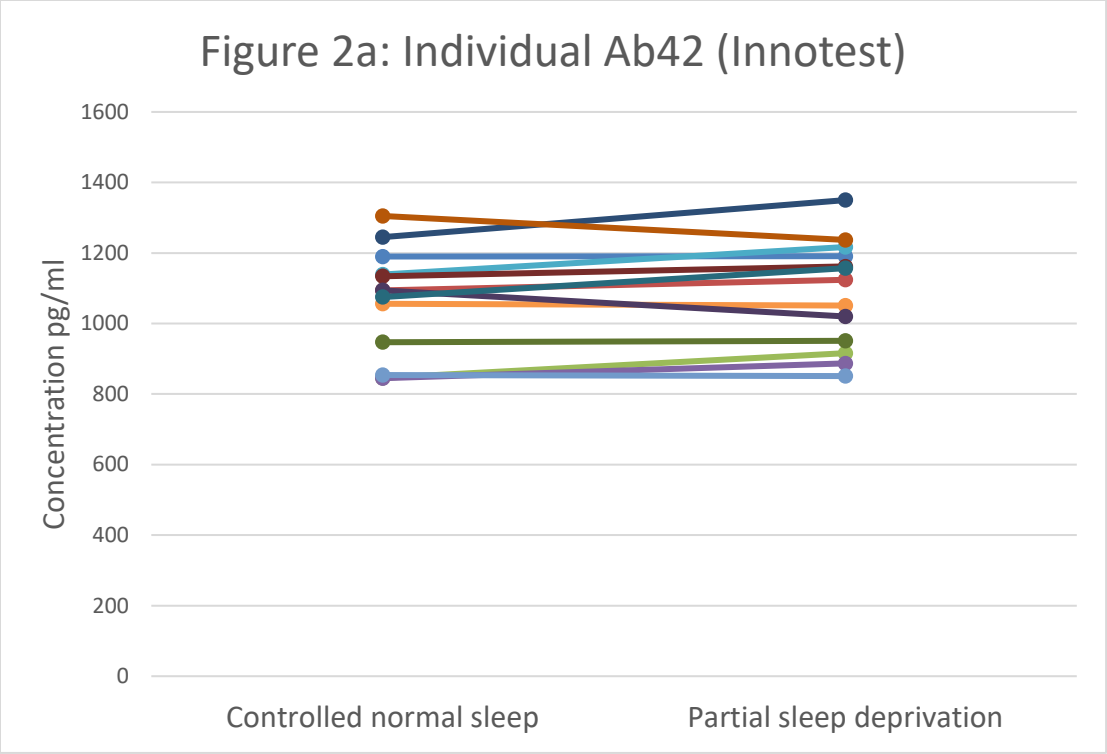


Figure 2a: Individual Ab42 (Innotest)
Concentration pg/ml
1600
1400
1200
1000
800
600
400
200
0
Controlled normal sleep
Partial sleep deprivation

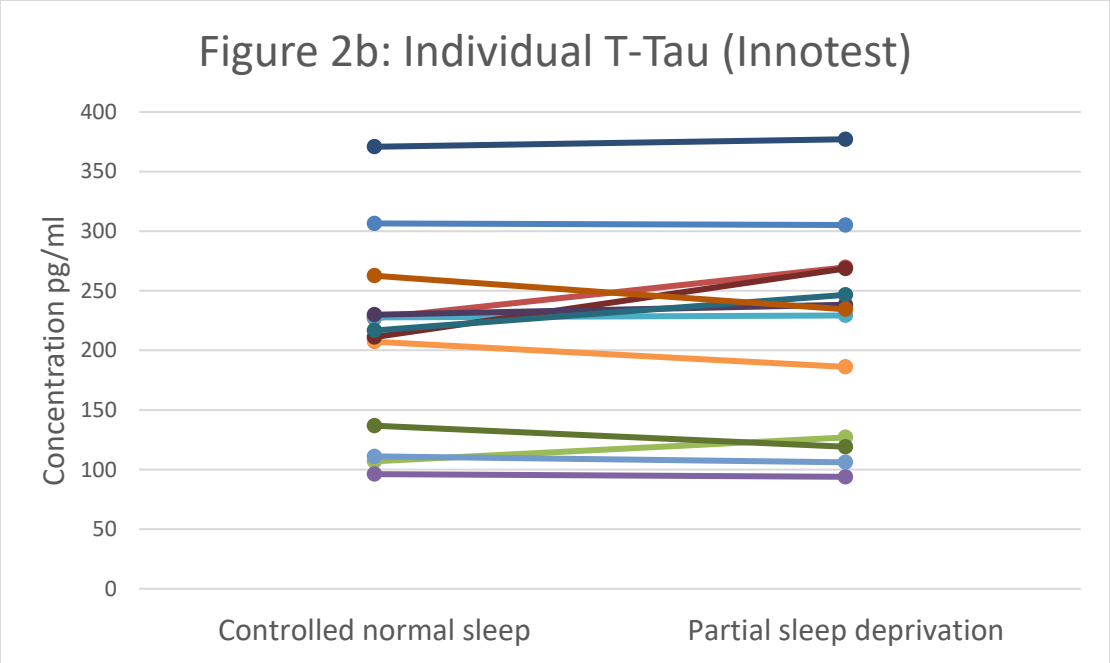
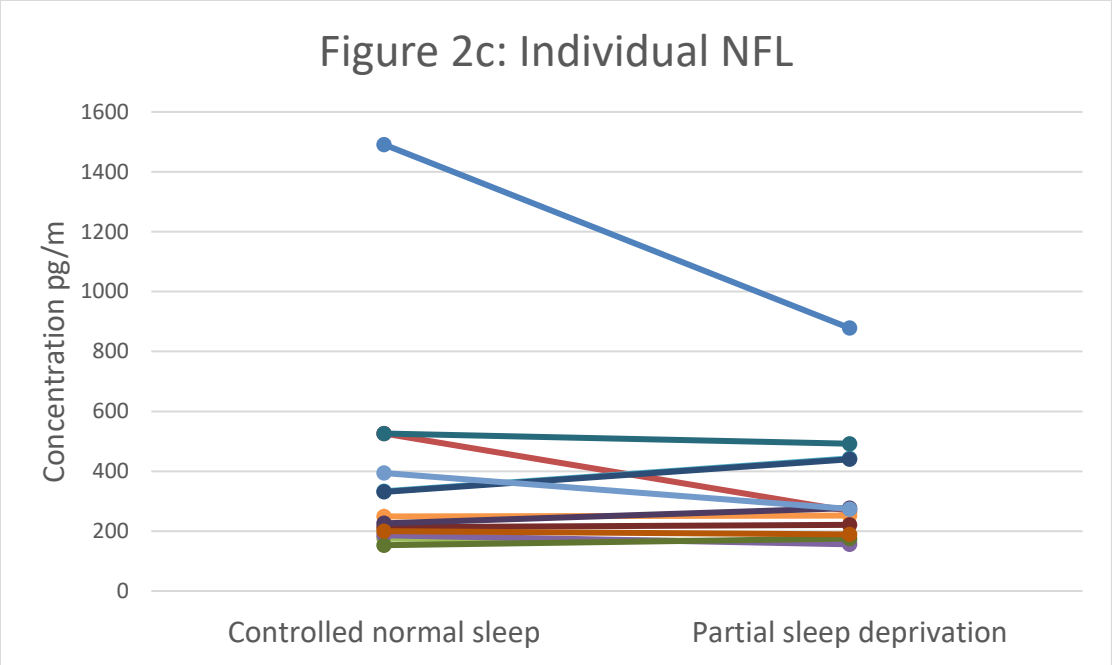


Figure 2b: Individual T-Tau (Innotest)
Concentration pg/ml
400
350
300
250
200
150
100
50
0
Controlled normal sleep
Partial sleep deprivation



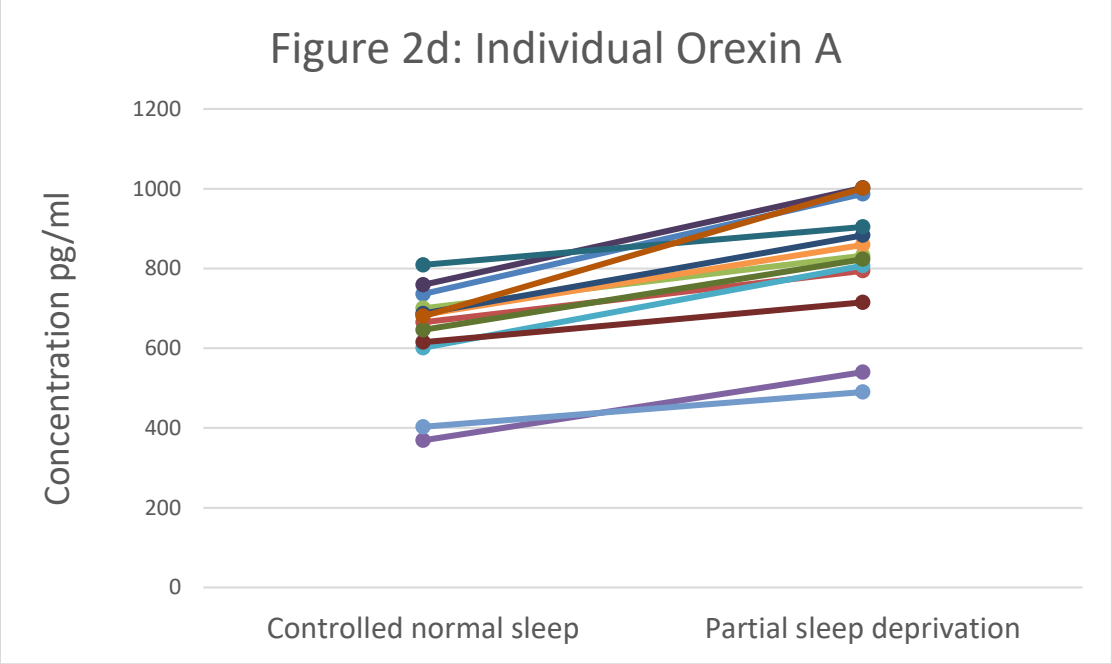


Figure 2a-d. Individual test subject CSF concentration of Aβ42, Tau, NFL and Orexin A. Controlled sleep vs. partial sleep deprivation.

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)

Abbreviations: BMI, body mass index. BP, blood pressure. ESS, Epworth Sleepiness Scale.

Table 2. Overview of Sleep data		Mean (SD)	
Variable	Controlled Sleep (N=13)	Partial Sleep Deprivation (N=13)	*P* Value
PSG variable, min			
TST	438.3 (27.7)	231.3 (4.4)	*0.02[a]
NREM stage 1	28.4 (12.9)	9.0 (5.8)	*0.02[a]
NREM stage 2	204.3 (28.1)	73.4 (15.7)	*0.02[a]
SWS/NREM stage 3	91.6 (21.5)	86.9 (16.2)	0.347[a]
REM	114.0 (21.4)	62.0 (8.2)	*0.02[a]
Actigraphic variable, min			
TST	482.7 (46.8)	230,00 (56,2)	*0.02[a]

Abbreviations: PSG, polysomnography. TST, total sleep time. NREM, non-rapid eye movement sleep. SWS, slow wave sleep. REM, rapid eye movement sleep.

[a] P-values represent within group (the same subjects 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

Table 3. CSF biomarker data. Baseline (CS) vs PSD		Mean (SD)		
Variable	Baseline (CS) (N=13)	Partial Sleep Deprivation (N=13)	*P* Value	Prolonged PSD (N=4)
CSF Value				
Orexin, pg/mL	642.6 (127.0)	818.2 (159.6)	**0.001[d]	771.3 (188.0)
Monoamine metabolites				
HVA, nmol/L	174.2 (65.5)	184.2 (63.3)	0.53[d]	177.8 (33.1)
5-HIAA, nmol/L	79.6 (25.7)	79.2 (18.0)	0.97[d]	77.3 (18.4)
MHPG, nmol/L	39.8 (6.5)	39.7 (7.1)	0.96[d]	38.8 (10.0)
Amyloid and associated biomarkers				
T-Tau, innotest[a], pg/mL	208.5 (80.5)	215.5 (85.0)	0.44[d]	188.2 (55.0)
P-Tau, innotest[a], pg/mL	38.1 (12.8)	39.4 (12.8)	0.12[d]	37.3 (8.7)
Aβ42, innotest[a], pg/mL	1063.4 (150.7)	1085.7 (152.9)	0.13[d]	986.0 (149.7)
Aβ38, MSD[b], pg/mL	2551.5 (710.2)	2639.7 (768.6)	0.46[d]	2370.8 (602.0)
Aβ40, MSD[b], pg/mL	7242.4 (1695.0)	7432.4 (1640.6)	0.35[d]	6594.6 (1410.8)
Aβ42, MSD[b], pg/mL	891.2 (240.7)	912.7 (247.1)	0.38[d]	800.6 (196.0)
Aβ38, Adx[c], pg/mL	2004.3 (512.3)	2048.4 (610.8)	0.43[d]	1845.7 (467.2)
Aβ40, Adx[c], pg/mL	7044 (2214.6)	7514.6 (2177.3)	0.09[d]	6452.4 (1870.8)
Aβ42, Adx[c], pg/mL	1042.5 (300.3)	1035.7 (309.9)	0.97[d]	914.3 (263.8)
T-Tau, Adx[c], pg/mL	206.8 (60.0)	212.9 (73.4)	0.62[d]	181.8 (34.0)
Neuron derived biomarkers				
NFL, pg/mL	384.8 (355.2)	325.2 (200.2)	0.46[d]	440.3 (289.1)
NSE, ng/mL	5.5 (1.6)	5.7 (1.9)	0.36[d]	5.0 (1.8)
FABP, ng/mL	3.5 (1.4)	3.5 (1.5)	0.50[d]	3.25 (0.8)
Astrocyte/Microglial derived biomarkers				
GFAB, pg/mL	187.6 (73.5)	182.8 (70.4)	0.80[d]	176.1 (79.6)
S100B, pg/mL	0.7 (0.2)	0.7 (0.1)	0.44[d]	0.7 (0.2)
YKL-40, pg/mL	56474.4 (26285.4)	56718.5 (24812.4)	0.92[d]	50120.9 (28928.7)

Abbreviations: 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-Tau, total Tau. P-Tau, phosporylated Tau. NFL, neurofilament light. NSE, neuron specific enolase. GFAP, glial fibrillary acidic protein. S100-B, calcium-binding protein B. YKL-40, chitinase-3-like protein.

[a]Fujirebio Innotest ELISA. [b]Aβ peptide panel 6E10 MSD ELISA. [c]Euroimmun Adx ELISA.

[d]P-values represent within group (the same subjects exposed to two sleep conditions) differences for the controlled sleep period samples compared with the partial sleep deprivation samples.