Acute sleep deprivation increases serum levels of neuron-specific enolase (NSE) and S100 calcium binding protein B (S-100B) in healthy young men

Running title: Acute sleep deprivation and neurodegeneration

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The authors have nothing to disclose.

The study is registered with ClinicalTrials.gov

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Abstract

Study objectives: To investigate whether total sleep deprivation (TSD) affects circulating concentrations of neuron-specific enolase (NSE) and S100 calcium binding protein B (S-100B) in humans. These factors are usually found in the cytoplasm of neurons and glia cells. Increasing concentrations of these factors in blood may be therefore indicative for either neuronal damage, impaired blood brain barrier function, or both. In addition, amyloid β (Aβ) peptides 1-42 and 1-40 were measured in plasma to calculate their ratio. A reduced plasma ratio of Aβ peptides 1-42 to 1-40 is considered an indirect measure of increased deposition of Aβ 1-42 peptide in the brain.

Design: Subjects participated in two conditions (including either 8-h of nocturnal sleep (2230-0630) or TSD). Fasting blood samples were drawn before and after sleep interventions (1930 and 0730, respectively).

Setting: Sleep laboratory.

Participants: 15 healthy young men.

Results: TSD increased morning serum levels of NSE (P=0.002) and S-100B (P=0.02) by approximately 20%, compared with values obtained after a night of sleep. In contrast, the ratio of Aβ peptides 1-42 to 1-40 did not differ between the sleep interventions.

Conclusions: Future studies in which both serum and cerebrospinal fluid are sampled after sleep loss should elucidate whether the increase in serum NSE and S-100B is primarily caused by neuronal damage, impaired blood brain barrier function, or is just a consequence of increased gene expression in non-neuronal cells, such as leukocytes.

Key words: sleep loss, sleep, neuron-specific enolase, S100 calcium binding protein B, amyloid beta

Introduction

With increasing duration of wakefulness, there is an increase in cerebrospinal fluid (CSF) concentrations of β-amyloid (Aβ) peptides [1-3]. In contrast, CSF concentrations of Aβ peptides decrease during sleep, i.e. a period during which the brain is minimally sensitive to environmental factors. As the production of Aβ peptides in the brain is intimately linked to neuronal activity [4,5], these findings suggest that nocturnal sleep may function as an offline period (i.e. a period with low sensory input) during which the brain recovers from daytime Aβ peptide accumulation. The aggregation of these peptides is hypothesized to be linked to neurodegenerative processes, most notably in Alzheimer's disease [6].

Neuronal damage has also been found to be indicated by elevations of two other neurochemical markers: neuron-specific enolase (NSE) - an enzyme found in all neurons [7] - and S100 calcium binding protein B (S-100B) – a protein which is mainly found in the glial cells of the peripheral and central nervous system [8]. However, as of yet, evidence to support that an acute disruption of the sleep-wake cycle affects circulating concentrations of these markers of neuronal damage in healthy young men is lacking. Thus, in the present study, we assessed circulating concentrations of Aβ peptides 1-42 and 1-40, NSE, and S-100B in 15 healthy young men, both before and after a night of either normal-duration sleep or total sleep deprivation.

Materials and Methods

Participants. Fifteen healthy Caucasian male subjects, all non-smokers, participated in the study. Participants were 23.3 ± 0.9 years old and had a mean body mass index of 23.4 ± 0.6 kg/m2. Exclusion criteria were as follows: night shift work or a transmeridian travel in the previous three months, a reported current or history of physical or psychiatric disorders, or if subjects were currently taking medication for any such condition. An interview prior to the experiment

ensured that all subjects enrolled in the study reported to have a normal sleep–wake rhythm, i.e. ~8 h per night, bedtime between 2230 and 2330 and wake up time between 0630 and 0730 on working days, and not more than 2 hours day-to-day variability with regards to their sleep duration. All participants gave written informed consent. Experimental procedures were in accordance with the Helsinki Declaration and were approved by the Regional Ethical Review Board in Uppsala. The current study is registered with ClinicalTrials.gov.

Study design and procedure. Each subject participated in two conditions (total sleep deprivation (TSD) and Sleep) spaced apart by 4 weeks. The order of conditions was counterbalanced across subjects. Experimental sessions were carried out on working days, and started with a 28.5 hours baseline period, starting at 1800. Following a baseline night in which the participants had an 8-hour sleep opportunity between 2230 and 0630 (i.e. resembling their sleep patterns on working days), they were provided with standardized meals during the following baseline day. Participants partook in two supervised 30-minute walks at 1000 and 1500. The baseline day was followed by the intervention night, with either sleep between 2230 and 0630, or TSD. Participants were blinded to the sleep intervention (i.e. sleep or TSD) until 2000 hours of the baseline day. Polysomnography was performed by use of Embla A10 recorders (Flaga hf, Reykjavik, Iceland) and comprised electroencephalography (EEG; Fp2-A1, C3-A2), electrooculography (EOG), and electromyography (EMG). An experienced scorer blinded to the study hypothesis scored sleep stages according to standard criteria [9]. Briefly, the sleep recordings were divided into epochs of 30 seconds. Each epoch was then scored as either wakefulness, rapid eye movement (REM) sleep, stage 1 sleep, stage 2 sleep, or slow wave sleep (i.e. sum of stage 3 and stage 4 sleep). In the TSD condition, a selection of books, games and movies was accessible for the participants. Also, lights were on in the TSD condition (~300 lux). Water was provided

ad libitum throughout the night, but no food intake was allowed. During each experimental session, participants were under supervision of the experimenter.

Laboratory assessments. On each of the two experimental conditions, fasting blood samples were drawn at 1930 in the evening of the baseline day and 12 hours later at 0730, i.e. following either one night of sleep or nocturnal wakefulness. Blood was collected using both serum separator tubes and plasma separator tubes (Becton, Dickinson and Company; Franklin Lakes, NJ, USA). Collected blood was allowed to clot for 30 minutes, followed by centrifugation at 2000 RPMs and 4° C for 10 minutes. Following its separation, ~1.5 ml serum and ~1.5 ml plasma for each subject were immediately frozen and stored until analysis at -80° C. Serum levels of NSE and S-100B (required serum volume: 500 µl) were measured using the Modular system (Cobas E601) and NSE and S-100B reagent kits (Roche Diagnostics, Basel, Switzerland). One subject from an initial sample of 16 subjects was excluded from analysis in the present study as his serum concentrations of NSE/S-100B after sleep loss were ~4 standard deviations higher than respective mean concentrations. Plasma concentrations of Aβ peptides 1-40 and 1-42 (required plasma volume: 500 µl) were assessed simultaneously using a highly sensitive Luminex xMAP® based method (INNO-BIA plasma Aβ forms; Innogenetics). Samples from each individual were analyzed on the same plate. Concentrations were determined using standard curves with the lowest detection point for Aβ40 (18pg/ml) and Aβ42 (15 pg/ml). The measurements were performed on a Luminex®200 analyzer (Luminex Corporation, Austin, TX, USA). All samples were analyzed in duplicate. Previous studies have shown that the ratio, rather than its single components, is the best predictor of developing Alzheimer's disease [10]. Thus, the ratio was analyzed herein.

Statistical analysis. SPSS version 17.0 (SPSS Inc, Chicago, IL) was used for all statistical analyses. NSE, S-100B and the ratio of Aβ 1-42 to Aβ 1-40 were separately analyzed using repeated measures ANOVA, with the within-subject factors '*Sleep*' (i.e. Sleep versus TSD condition) and '*Time*' (i.e. evening versus morning blood sample). All post-hoc tests were performed using pairwise student's *t*-tests. All variables are presented as means (± SEM). $P<0.05$ was considered significant.

Results

Acute sleep loss increases morning serum levels of NSE and S-100B. Repeated measures ANOVA yielded an interaction effect for serum NSE concentrations ($F(1,14)=5.10$, $p=0.04$ for the *Sleep*Time* interaction). While there was no difference in serum concentrations of NSE in the evening before sleep interventions, pairwise *t* test comparisons showed that serum concentration of NSE assessed in the morning after sleep deprivation was about ~20% higher, than those measured after one night of laboratory sleep ($P=0.002$; **Figure 1, left panel**). For serum concentrations of S-100B, repeated measures ANOVA yielded a main effect for *Sleep* ($F(1,14)=7.53$, $p=0.02$). In contrast, no main *Time* effect and no significant interaction were found. Subsequent pairwise *t* test comparisons revealed no differences in serum concentrations of S-100B before sleep intervention, but a significant ~20% increase of serum concentrations of S-100B upon sleep loss as compared with sleep ($P=0.02$; **Figure 1, middle panel**).

Acute sleep loss does not affect the plasma ratio of Aβ peptides 1-42 to 1-40. While there was an overnight decline by about ~5.5% in the plasma ratio of Aβ 1-42/1-40 ($F(1,11)=11.23$, $P=0.006$ for the ANOVA main *Time* effect), no main *Sleep* effect and no significant interaction were found (**Figure 1, right panel**).

Sleep. Sleep length and quality in the sleep condition were typical for laboratory condition (in min [% total time in bed]: total sleep time, 441 ± 6 [92 = sleep efficiency]; wake, 31 ± 4 [6]; sleep stage 1, 6 ± 1 [1]; sleep stage 2, 222 ± 11 [46]; slow wave sleep, 111 ± 5 [23]; REM sleep, 102 ± 9 [21].

Discussion

Here we demonstrate in healthy young men that a single night of sleep loss increases morning serum concentrations of NSE and S-100B by about ~20 %, relative to values obtained after one night of sleep. These factors are usually found in the cytoplasm of neurons and glia cells [11-14]. These findings therefore suggest that a good night's sleep may possess neuroprotective function in humans, as has also been suggested by others [15,16].

During a normal sleep-wake cycle, sleep represents a period during which brain glucose metabolism drops by ~30%, compared with values obtained during wakefulness [17]. One reason for this sleep-related drop in central nervous system energy expenditure might be that the thalamic relay of environmental information to sensory cortical areas is dampened [18]. In contrast, during nocturnal wakefulness, this relay of sensory information is nearly as high as it is during daytime [18]. Substrate oxidation ultimately leads to the production of reactive oxygen species (ROS), such as hydrogen peroxide [19]. Previous experiments have demonstrated that ROS can damage neurons and even induce cell death [20]. With this in mind, the increase in morning serum concentrations of NSE and S-100B observed after a single night of sleep loss in our participants might be caused by an increased nocturnal ROS production in the brain.

Recently, it has been demonstrated that acute sleep deprivation increases CSF concentrations of Aβ peptides in mice [1]. Considering that Aβ peptide accumulation in the brain extracellular space is a hallmark of Alzheimer's disease [6], this finding suggests that poor sleep patterns, if chronic,

may increase the risk of developing Alzheimer's disease. Thus, in the present study, we measured the plasma ratio of Aβ peptides 1-42 to 1-40. A recent meta-analysis has shown that a low plasma ratio of Aβ peptides 1-42 to 1-40 is linked to an increased risk to develop Alzheimer's disease [10], most likely as a by-product of an increased deposition of Aβ 1-42 peptide in the brain. However, in our study, this ratio did not differ between the sleep deprivation and sleep conditions. Several reasons may have masked a possible effect of sleep loss on this ratio. First, longer periods of sleep deprivation might be needed to affect the plasma ratio of Aβ peptides 1-42 to 1-40. Second, plasma may be less sensitive than CSF to reflect the effects of acute sleep deprivation on Aβ peptides.

When interpreting our results, several limitations should be kept in mind. Lights were on in the TSD condition but not in the sleep condition (~300 lux versus darkness). Thus, it cannot be ruled out that light exposure may have contributed to the sleep-deprivation-induced increase in serum levels of S-100B and NSE. Another limitation is that the increase in serum concentrations of NSE and S-100B in the morning after sleep loss might be induced by an impaired blood-brain barrier (BBB) function (i.e., as a result of leakage from the CSF) [21], rather than a consequence of increased nocturnal neuronal damage. To test this hypothesis, future studies could measure beta-trace protein, which is highly enriched in the CNS and CSF, and has been proposed as biomarker to diagnose CSF leakage after trauma or surgery [22]. Alternatively, both serum and CSF could be sampled after sleep loss. This would allow elucidating if the increase in serum NSE and S-100B is primarily caused by neuronal damage, impaired blood-brain barrier function, or is just a consequence of increased gene expression in non-neuronal cells, such as leukocytes [23]. Such CSF measures would also help to draw definite conclusions about the effects of acute sleep loss on Aβ peptide metabolism.

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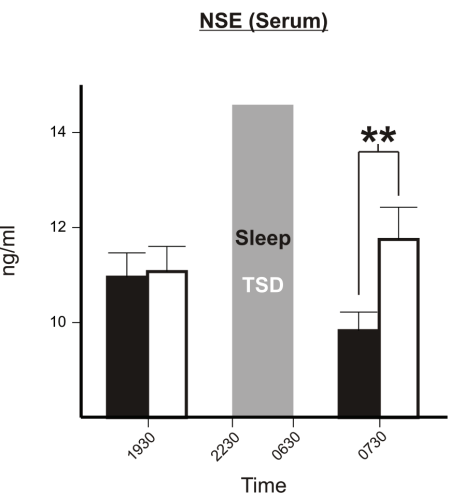
Figure 1. Serum concentrations of neuron-specific enolase and S-100B, as well as the plasma ratio of amyloid β peptides 1-42 to Aβ 1-40 after one night of either sleep or total sleep deprivation. On each of the two experimental conditions, blood was sampled at 1930 in the evening preceding the experimental night of sleep (lights off: 2230-0630; black) or nocturnal wakefulness (white), followed by fasting blood samples at 0730 the next morning. *P < 0.05 and **P < 0.01. Data are means ± SEM. Abbreviations: Aβ, amyloid β, NSE, neuron-specific enolase; TSD, total sleep deprivation.


NSE (Serum)
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Sleep
TSD
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2230
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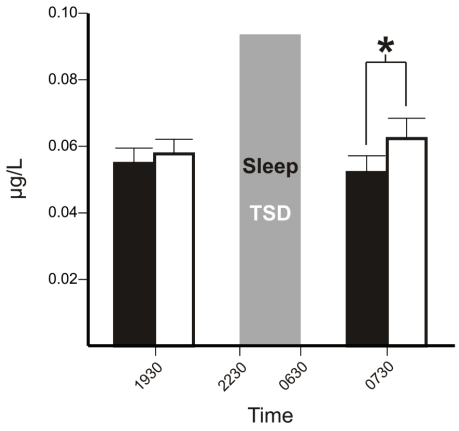
Time


S100B (Serum)
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Sleep
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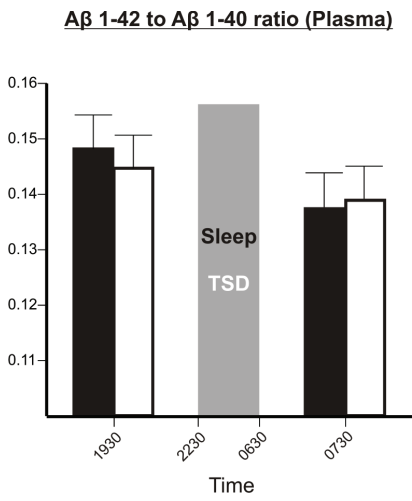
Aβ 1-42 to Aβ 1-40 ratio (Plasma)
0.16
0.15
0.14
0.13
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0.11
Sleep
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Time