Sleep deprivation and organic effects in the central nervous system

Dynamics of biomarkers in cerebrospinal fluid

Master thesis in Medicine

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Table of Contents

Abstract

Master Thesis , Programme in Medicine

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- Dynamics of biomarkers in cerebrospinal fluid

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Objectives This study addresses potential CSF and blood biomarkers that may be affected by controlled sleep deprivation in healthy human subjects. We hypothesize that relevant biomarkers might be found among those conventionally related to some forms of neuronal degeneration and damage.

Methods Study participants were subjected to one period of sleep deprivation (5 nights with < 4 hrs of sleep) and one period of controlled normal sleep (5 nights with 8 hrs in bed). Sleep was monitored by polysomnography and actigraphy. CSF was collected by a routine lumbar puncture in the morning following each period. CSF was also collected 3 days after the sleep deprivation period (recovery). CSF analysis included total-tau (TT), phospho-tau (PT), amyloid β42 (Aβ42) and orexin (OX). Four healthy volunteers with self-reported normal sleep and no daytime excess sleepiness were included in the current interim analysis.

Results CSF concentrations of TT, PT and Aβ42 remained relatively unchanged from the normal sleep to the sleep-deprived state. In contrast, there was a pronounced increase in the concentration of TT (270 and 605 ng/L), PT (40 and 82 ng/L) and Aβ42 (1001 and 1628 ng/L) following three days of recovery. OX, on the other hand, followed an expected pattern (from 754 to 857 and 638

pg/mL, respectively) which corroborates that the observed changes in TT, PT and Aβ42 were unrelated to possible changes in CSF dynamics/volumes.

Conclusions Recovery sleep following brief sleep deprivation induced pronounced changes in CSF concentrations of TT, PT and Aβ42. These markers have been associated with Alzheimer's disease but their role in normal physiology is largely unknown. The observed changes may shed further light on sleep-associated physiological effects in the brain, particularly during recovery from sleep loss.

Key words Alzheimer's disease, amyloid β, cerebrospinal fluid, sleep, sleep-deprivation, tau.

Background

Today we have the ability to analyse a wide range of biomarkers in cerebrospinal fluid. Some of these are specific for nerve cell damage and glial cell damage (1). There is also a great amount of research indicating that sleep is associated with a number of health issues such as depression, cancer and obesity (2-5). Of certain interest is that an increased removal of Alzheimer-associated amyloid β (Aβ) has recently been shown in live mice during sleep (6). An overview of available literature shows a lack of knowledge on the specific effects of sleep deprivation on the human neuron.

Sleep physiology

Why sleep?

Sleep is an essential function in all animals. No species of animals have been found that, with certainty, do not sleep (7). Even species who are dependent on maintaining consciousness at all times, such as dolphins, have found ways around this by letting one cerebral hemisphere sleep at a time (8). Rats that are kept awake for prolonged periods shows another aspect of how vital sleep is as they will eventually die in a septic like state (9). It is hard to overestimate the importance of sleep, but it is still largely unclear why we sleep. Fortunately recent studies have shed some new light on this question.

The human central nervous system lacks lymphatic drainage in the way other tissues does. Instead it is dependent on the glymphatic system for this function. In 2013 L. Nedergaard et al (6) showed, in mice, that the exchange between the neuronal interstitial space and cerebrospinal fluid was increased during sleep by enhanced glymphatic activity. In their experiment they saw an increase in volume of the interstitial space by 60% during sleep. Elevated clearance of amyloid β (Aβ) was also observed. Sleep was induced by anaesthesia which suggests that it is not a circadian

effect but directly associated with sleep.

There is evidence that sleep has both short- and long-term effects on the immune system. Tufik et al has shown that sleep deprived rats will have a 20% decreased volume of white blood cells after one night of total sleep deprivation (10). The effect were seen in all used variables, including lymphocytes, monocytes, neutrophils and spleen size. Mice will also have a smaller chance of surviving sepsis if not allowed to sleep (11). Further evidence of just how closely connected sleep is to the immune system and inflammation, is the fact that the well known immune modulating cytokine TNF (tumor necrosis factor) is in itself a sleep inducer (12).

A recent study on human test subjects found that one week of sleep deprivation caused down-regulation of 444 genes and up-regulation of another 267 genes measured by detecting RNA in whole blood (13). The sleep-deprivation protocol used resulted in 5.7 hours of sleep per night during the deprived period versus 8.5 hours during the sleep extended period. Many of the affected genes are related to human immune function (14). This is a profound effect on human transciptome even within what could be considered normal, or at least close to normal, sleeping patterns.

There are many more, established or possible, reasons for the evolution of sleep; too many to be described in detail here, though some need a very brief mentioning:

- Sleep is connected to endocrine processes such as growth hormone, cortisol and thyroid -stimulating hormone secretion (15, 16).
- Human metabolism is affected by sleep. The effects is intricate and not fully understood, but lack of sleep decrease insulin sensitivity and affects lipid metabolism (14, 16, 17) .
- Sleep is important for memory processing and consolidation (18).
- It has been theorized that REM sleep in particular is important for ontogenesis (19).

In humans there are three main variables that constitutes normal sleep: Sleep duration, sleep structure and timing in respect to circadian rhythm (20). Quantifying normal sleep is a hard, not to say impossible task, since there are so many different variables and there is such great individual variability in all of them (20). Apart from genetic and environmental impact on these variables, ageing will also affect them, with a relative increase in REM (rapid eye movement) sleep, decreased length of sleep and changed circadian rhythm (20, 21) .

As always one will also have to consider what endpoint is of the most importance when we try to find out what is optimal or normal sleep. Different endpoints such as mortality, morbidity and cognitive performance produce different results. For example; studies have shown that relatively short sleep, six to seven hours, is associated with the lowest mortality (22, 23) but recent sleep recommendations still states that seven to eight hours of sleep is optimal for adults (24).

Sleep structure describes the stages of sleep and their length. There are four stages of sleep (20, 25). They are designated N1, N2, N3 and REM, where N denotes nonREM. During normal circumstances the brain will go through these in the aforementioned order. The first cycle of the night will take approximately 90 minutes and every cycle thereafter will be progressively shorter. Different stages seem to serve different purposes. The amount of deep sleep (N3 or slow wave

Illustration 1 Hypnogram showing a normal sleep structure.
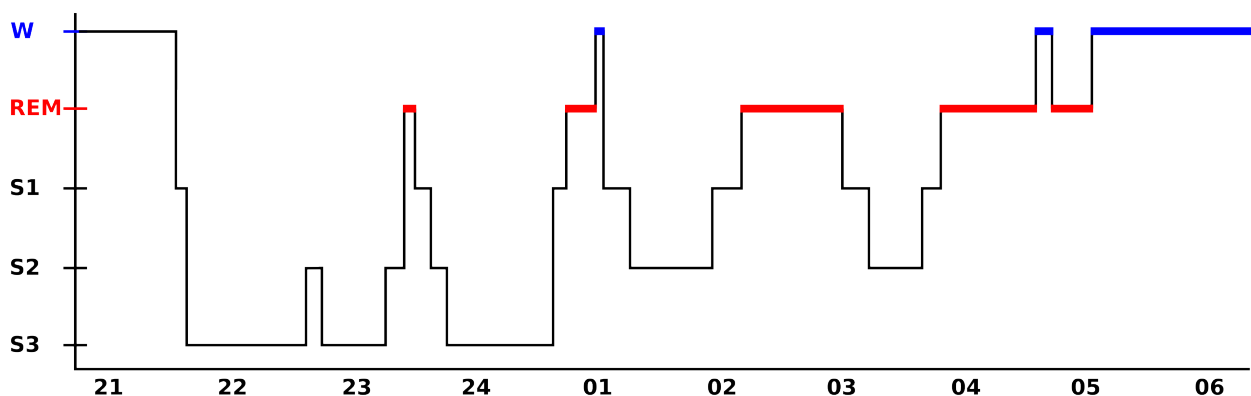
S1-S3 denotes nonREM stages 1-3.

sleep) will affect your feeling of sleepiness the most but REM sleep on the other hand is more important for memory processing.

In sleep science terminology, timing is the time of sleep induction in relation to the subject's circadian rhythm. Bad timing will affect your sleep structure and make your sleep less effective with decreased amount of slow wave sleep (26). Negative health effects of night shift work are evidence of the importance of timing. Night shift workers who maintain a constant day/night rhythm and get a sufficient amount of sleep will still have a higher risk for disease (26).

One way to look at sleep requirement is the point when there is no daytime sleepiness. But this is not without problems either, since there are sleep disorders, such as obstructive sleep apnea (OSA), where some patients report no daytime sleepiness even though they may have a manifest disease which impacts their health. As with most biological measurements we are left with a large variety of what is normal. Sleepiness or the lack thereof is still the clinically most relevant marker for healthy sleep habits.

Sleep deprivation and disease

There are health conditions that have been linked to sleep, or lack thereof, in almost every field of medicine. Examples are autoimmune disorders (27), cancer (3), cardiovascular disease (17) and psychiatric conditions (2, 5) . Considering the effect of sleep on genetic expression, metabolism and our immune system, this should come as no surprise. The exact pathways and if sleep disturbance is the cause or the symptom are not always clear. One example of this is Alzheimer's disease; a progressive memory disorder characterized by neurodegeneration and accumulation of Aβ and phosphorylated tau in the brain. Some recent studies suggest that many common sleep disorders may precede clinical disease (28). Also, lack of good sleep seems to be a risk factor for AD in itself. In later stages of AD, sleep disorders are very common, probably because of degeneration of neurons involved in sleep regulation.

Biomarkers

For the pilot part of this study we will examine four main biomarkers, and some variants of these, in cerebrospinal fluid. Aβ and Tau were chosen because of the connection to AD. S-100B and Neurofilament light, because of our initial hypothesis that sleep deprivation involves stress to the central nervous system, and these are good candidates to reflect this. Below is a very brief introduction to these biomarkers together with an slightly more detailed explanation to why each of these was chosen. Many more biomarkers, both in blood and CSF, will be analysed when we have completed the protocol for all 16 participants.

Amyloid beta

Amyloid beta (Aβ) is a class of 36 to 43 amino acids long peptides, the 42 amino acid form of which is the main component of amyloid plaques found in Alzheimer patients' brains (1, 29). Aβ is created by enzymatic activity, where amyloid precursor protein (APP) is cut into beta peptides. If these peptides get miss-folded they may acquire the ability to affect other beta peptides to miss fold in the same manner, much like prion disease. Not only will these have similar form but they will also gain the ability to adhere to each other and the build up of plaques can begin. The normal function of beta amyloid is still under investigation.

What makes amyloid beta interesting from a sleep deprivation perspective is the connection between sleep and Alzheimer's disease and the recent finding that amyloid beta clearance from the central nervous system is increased during sleep (6). Of all the amyloid peptides, we have chosen beta 42 because a previous study showed the most significant difference, in normal sleep versus sleep deprived subjects, with this peptide (30). It is also believed to be the main culprit behind the creation of plaques (29).

Neurofilament light

The three forms of neurofilament (light, medium and heavy) are a major part of the neuronal cytoskeleton, especially in large myelinated axons (31) . They belong to the intermediate filament family and provide structural support for the axon and decide its thickness. The diameter will decide the conductive velocity of the fibre (32). Neurofilaments are expressed soley in neuronal cells. Neurofilament light (NFL) is the most common and smallest of the three types of neurofilament and is the only one able to form polymeres on it's own (33). Elevated concentration of NFL in CSF is associated with various neurodegenerative diseases such as amyotrophic lateral sclerosis, Alzheimer's disease (34) and Charcot-Marie-Tooth disease (35). NFL is already used clinically to asses neuronal degeneration and therefore it is of interest to this study.

S-100B

S-100 is a family of calcium-binding proteins. They are present in a wide range of cells but in the brain they are most prominent in astrocytes and oligodendrocytes. S100B is only expressed in two subtypes of astrocytes: one that surrounds blood vessels, another that expresses NG-2 and resides in the brain parenchyma and is believed to be a oligodendrocyte precursor (36, 37). Its functions are complex and include trophic effects at low concentrations, but toxic effects at higher concentrations. S100B has an effect on the neuronal cytoskeleton by inhibiting microtubule assembly, a regulating role in the cell cycle and it plays a part in axonal proliferation, among other things (36).

Clinically, S-100B can be used as a marker for neuronal damage. It has been shown that elevated levels of the protein is present after head trauma (38), in Alzheimer's disease (39, 40), Downs syndrome (40) and other neurological and somatic diseases. Of certain interest is that a recent study also found that S-100B levels in blood increase after one night of total sleep deprivation (41).

Tau is an important protein involved in stabilizing the neuronal microtubule (42) of the central nervous system. There are six isoforms of tau of which all are present in the brain. They are abundant in neurons but there are also low levels in other tissues and there is a small production in astrocytes and oligodendrocytes (43). Tau can affect tubular stability either by its isoform or by phosphorylation, which makes the protein detach from the microtubules.

In Alzheimer's disease it is hypothesized that Amyloid beta load will eventually lead to hyperphosphorylation and mis-folding of tau (1). This can make the otherwise soluble protein to form insoluble aggregates known as tangles. Tangles are a central part of Alzheimer's disease. Elevated levels of tau has also been detected after traumatic brain injury and is associated with poor outcome (44).

We have chosen to examine the CSF levels of tau and its phosphylated form in relation to sleep deprivation. They are of interest to sleep-deprivation because they are commonly used markers for neuronal injury and Alzheimer's disease progression (1), both of which we hypothesize are associated with sleep.

Aim

We aim to explore the possible effects of sleep deprivation on human brain physiology and function by studying cerebrospinal fluid and blood from sleep-deprived test subjects. We hypothesize that there is a connection between sleep deprivation and elevated levels of CSF biomarkers related with neurodegeneration and neuronal damage. We also hypothesize that this may contribute to the development of neurodegenerative disease such as Alzheimer's.

Material and Methods

Study population

This project was created as a pilot study for a larger project in which the author is still involved. Data will be collected from the first four study participants as a proof of concept, but research will carry on and include another 12 test subjects. In total, sixteen healthy test participants between the age of 20 and 40 and with no sleep disturbances, will be included. Subjects with a Body-Mass-index (BMI) above 30 kg/m2, continual use of medication or relevant chronic diseases will be excluded. Our definition of normal sleep pattern includes self reported normal bedtime < 00.00, regular morning awakening hours 06.00-09.00, habitual sleep duration of between 6.5-8.5 hours and absence of sleep disturbances (such as chronic insomnia/daytime sleepiness/narcolepsy).

The Epworth Sleepiness Scale (ESS) was used to further evaluate excess daytime sleepiness and a cut off score was set to <11. Which is a standard value commonly used in sleep deprivation protocols. Test subjects will stop using caffeine or nicotine at least two days prior to the study period. Use of central stimulating substances, by subjects, during the experiment, will lead to exclusion. Of the four subjects in our pilot, two were female and two were nicotine users.

Figure 2: Flowchart of participant inclusion
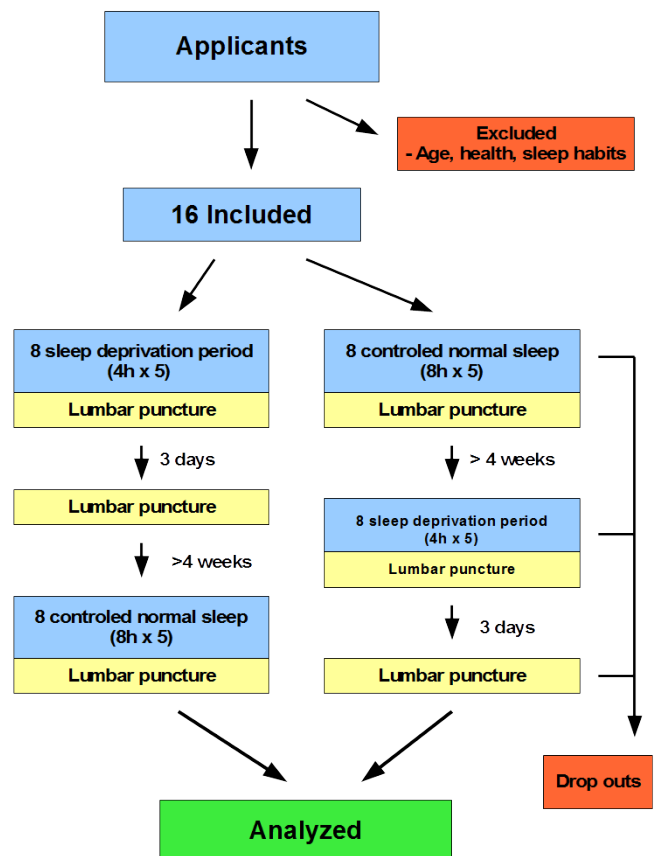
Sleep deprivation protocol

The participants will be subjected to a period of partial sleep deprivation (5 consecutive nights consisting of a maximum of four hours of sleep per night) with two baseline nights, consisting of eight hours total time in bed between 22.00-0800, prior to the sleep restriction period in accordance to a standardized protocol (45). The participants will also undergo a period of controlled normal sleep (five nights of eight hours total time spent in bed), thus making the test subjects their own controls. The two periods will be separated by at least four weeks of normal sleeping pattern without our influence to minimize the risk of interference. Half of the group of test subjects will have the sleep deprivation period prior to the period of controlled normal sleep whilst the other half will have the opposite arrangement. During the sleep-deprivation period participants were not allowed to bring their own food to the lab. They were instead offered one standardized meal (Frozen meal with <500 kcal) every night.

Sleep surveillance

During our study two different systems of sleep surveillance were used; polysomnography (PSG) and actigraphy. Multiple methods ensures data quality and provides technical redundancy.

PSG were used to retrieve data when subjects were in bed at the laboratory. The Actigraph were used for the complete period to ensure that protocol is followed both at our laboratory and when at home.

During the sleep restriction period subjects arrived at the ward at 10 pm every night. They were observed by staff while at the lab. Bedtime were set between 3 and 4 am and the PSG equipment were hooked up well in advance of bedtime. While in the controlled normal sleep period participants slept at the laboratory during the first and last night. The first night were spent at the lab to habituate and lessen the risk of the so called "first night effect".

Actigraphy

Actigraphy is a validated modality for testing total sleep time (46). The actigraph is a small device worn on the test subjects non dominant wrist, it will record data of ambient light and motion which will later be evaluated in a validated software. It is comparable, but not quite as precise, as the polysomnograph in the detection of sleep stages. We have chosen to use the "wGT3X-BT Monitor" for sleep surveillance and the Actilife software for data analysis, both are made by ActiGraph. Below is a an exampel of an automatic analysis with the Actilife software.

Figure 3: Visualization of sleep data retrieved from an actigraph
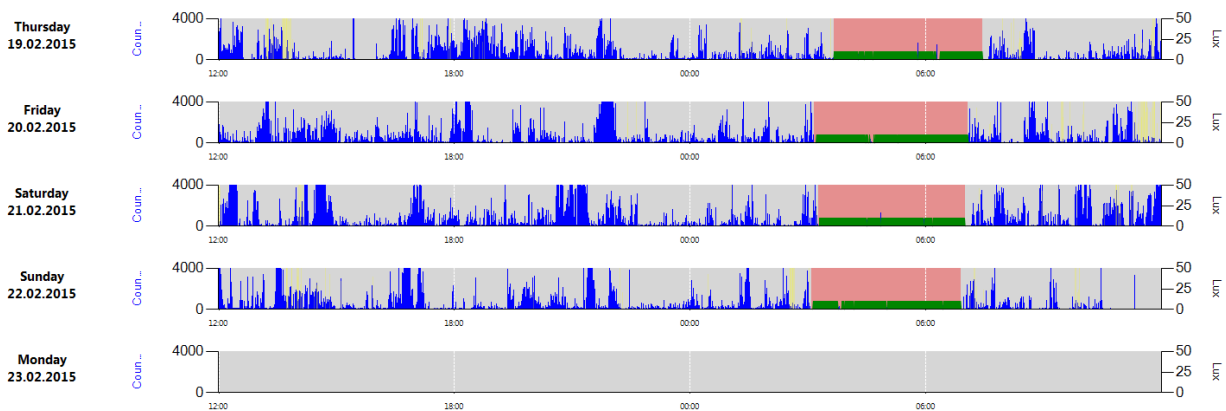
The shadowed area represents sleep as interpreted by the Actilife software

Polysomnography

Polysomnography (PSG) is the gold standard for in depth evaluation of sleep in test subjects. A PSG consists of a continuous recording of electrical activity in the brain (eeg), skeletal muscles (emg), eye muscles (eog) and the heart (ecg). Other modalities can also be added, such as pulse oxymetry and nasal air flow. Blinded data is later analysed by certified personel. Fig 3 shows how data is visualized when using PSG.

Figure 4: A polysomnographic recording of a testsubject experiencing slow wave sleep
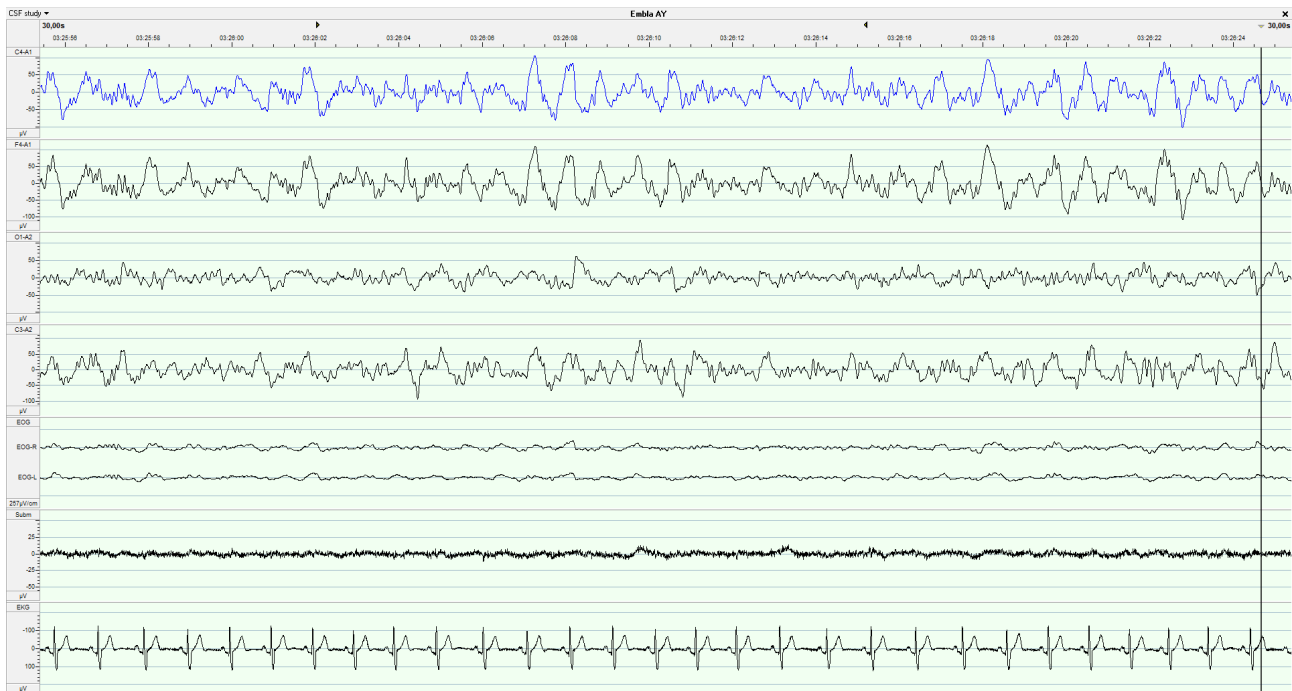
The top four rows represents different EEG projections. The following two rows shows eyemovements by EOG. Next is an EMG of the tounge and last is a single ECG projection.

Blood and CSF sampling

In the morning of the sixth day and another 3 days after the sleep restriction period, blood and CSF will be obtained from the participants by veni- and lumbar puncture respectively. CSF will also be retrieved after the period of controlled normal sleep (five nights of eight hours total time spent in bed), thus making the test subjects their own controls.

CSF (10-12 ml) will be collected in polypropylene tubes, centrifuged (1300 x g, 10 min), aliquoted and stored in 0.5 ml aliquots at -80°C pending analysis. Five ml blood will be collected in an EDTA tube for plasma preparation and another 5 ml will be collected in a gel tube for serum preparation. Following centrifugation (1300 x g, 10 min), plasma and serum will be aliquoted into 0.5 ml aliquots and stored at -80°C pending analysis.

Blood and CSF analysis

CSF total tau (T-tau, a marker of cortical axonal integrity), phospho-tau (P-tau, a marker of tau phosphorylation and tangle pathology), neurofilament light (NFL, a marker of subcortical axonal integrity), 42 amino acid long Aβ fragments (Aβ42, marker of amyloid metabolism), soluble , as well as and S100B (markers of astroglial activation/injury) will be analysed using commercial immunoassays. In addition to the CSF markers, serum samples will be analysed for T-tau and NFL using immunoassays on an ultra-sensitive single molecule array (Simoa) platform.

Data collection and statistical methods

Since the data for this report will include only four test subjects, statistical analysis will be futile. The data will be presented as is and variables will be looked upon separately and without calculation of correlation, significance etc.

Ethics

Lumbar puncture

The collection of cerebrospinal fluid by lumbar puncture is a well established method that involves few risks. The main concerns regarding this method is pain during the procedure and head ache that typically begins within one or two days after the procedure, and last another day or two. To minimize these risks we will strive to use the smallest possible diameter needles. The needles used will also be so called atraumatic needles, which will further lessen the risk of post puncture headache (47, 48) .

Sleep deprivation

The test subjects will be subjected to five days of restricted sleep. This will lead to discomfort in itself as the test subjects will experience sever sleepiness. There is also an increased risk of accidents involved since sleep deprivation decreases attention and reaction time. The test subjects will be informed of this risk and will be asked to avoid situations which demands full vigilance. Examples of such situations are driving a vehicle and operating heavy machinery.

Permissions

The study has been granted approval from the Regional Ethical Review Board in Gothenburg, Sweden. The Declaration of Helsinki and The Universal Declaration of Human Rights have been considered and respected in the designing and execution of this study.

Results

Table 1: CSF levels for each individual test subject and average biomarker levels in CSF including ratios between different time points.

Test subject A	NFL	s-100B	T Tau	P Tau	Aβ42*	Aβ42**	Orexin
Sleep extention	1569	0,684	345	52	951	993	728
Sleep deprivation	756	0,74	345	51	970	1018	957
Recovery	668	0,979	1009	128	2084	1246	598
Test subject B	NFL	s-100B	T Tau	P Tau	Aβ42*	Aβ42**	Orexin
Sleep extention	436	0,681	279	40	1040	995	614
Sleep deprivation	153	0,685	296	43	1164	1089	778
Recovery	162	0,71	669	85	1729	1232	602
Test subject C	NFL	s-100B	T Tau	P Tau	Aβ42*	Aβ42**	Orexin
Sleep extention	119	0,519	123	20	657	816	688
Sleep deprivation	157	0,537	150	23	734	932	734
Recovery	158	0,53	172	27	782	971	547
Test subject D	NFL	s-100B	T Tau	P Tau	Aβ42*	Aβ42**	Orexin
Sleep extention	158	0,609	300	46	1215	1124	985
Sleep deprivation	245	0,652	287	44	1135	1034	959
Recovery	198	0,654	570	87	1918	1268	804
Averages	NFL	s-100B	T Tau	P Tau	Aβ42*	Aβ42**	Orexin
Sleep extention	570,5	0,623	261,8	39,5	965,686	982,0	753,8
Sleep deprivation	327,8	0,654	269,5	40,3	1000,684	1018,3	857,0
Recovery	296,5	0,718	605,0	81,8	1628,144	1179,3	637,8
SD/SE ratio	0,57	1,05	1,03	1,02	1,04	1,04	1,14
Recovery/SD ratio	0,90	1,10	2,24	2,03	1,63	1,16	0,74

*amyloid-β peptide Triplex Assay **INNOTEST® β-AMYLOID immunoassay*

There were only small differences in CSF biomarker data between the normal sleep and sleep-deprivation time periods (insignificant with so few data points). However, following recovery sleep (three nights of unrestricted sleep after the sleep-deprivation period) considerable changes had occurred. Total- and phopsho-tau levels increased more than 2-fold in 3 out of 4 subjects. The trend was also seen in the the fourth subject but not quite as pronounced. Such levels are otherwise only seen in AD patients. CSF levels of the 42 amino acid long senile plaque-associated form of Aβ also

increased significantly. The relative increase of Aβ42 differed in an unexpected way between methods. Opposite changes were seen in the CSF levels of orexin (decreased levels after recovery sleep), corroborating that the tau and Aβ changes at this time point were not just reflecting changes in CSF dynamics/volumes. The difference that can be seen in NFL were not consistent between test subjects and both increased and decreased concentrations were observed. There is also some interesting changes in s-100B, but to small to draw any conclusions at this point.

Table 2: CSF concentration of selected biomarkers

Biomarker concentrations in CSF
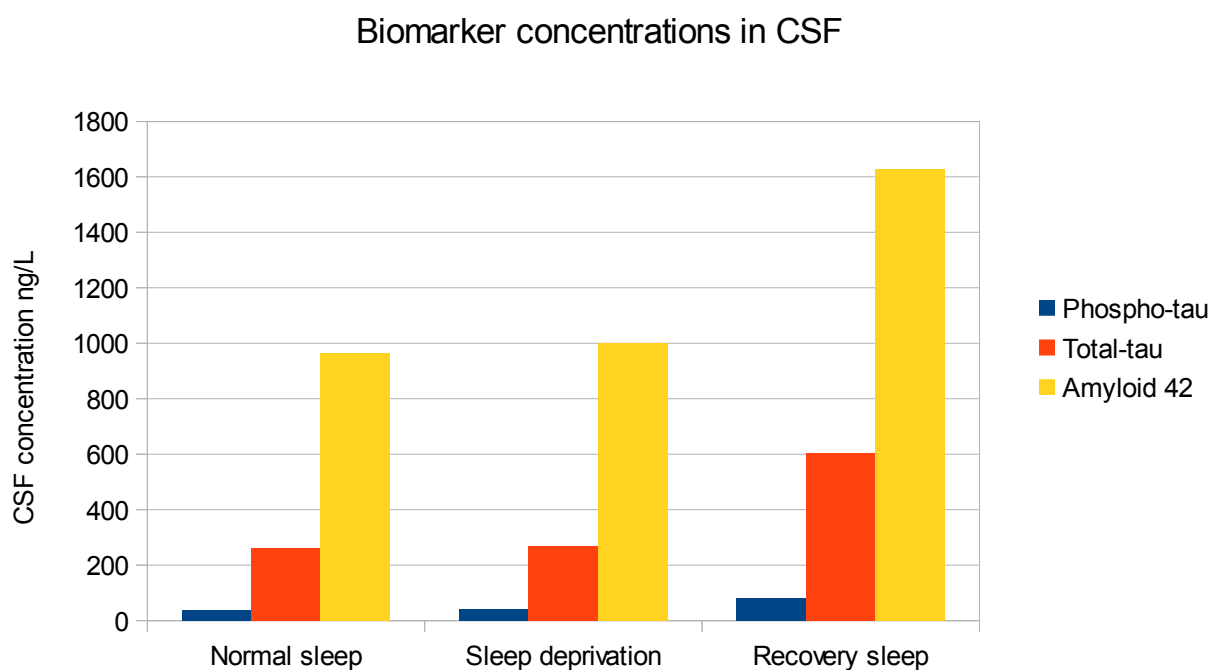
So far sleep data is limited to actigraphy and observation as the PSG recordings have not yet been analysed by a certified PSG technician. What we do have is Actilife software visualization of actigraph data. We have made both automatic and manual sleep time estimations. The exactness of our rudimentary analysis's is not enough to use for data comparison but is enough to confirm that protocol was mostly upheld. The only major departure from protocol was test subject D's last night of controlled normal sleep, were the test subject had trouble falling and staying asleep (While at the

sleep centre. Further discussion below). Unfortunately this is probably the night with the most impact on CSF chemistry. This explains the deviation in orexin levels of subject D compared to the other subjects. Some other departures from protocol were found but were minor and should not effect results. During the recovery period we only have self reported data of sleep duration. Interestingly all subjects reported between 29 and 30 hours of sleep in the recovery period (between 9.7 hrs to 10 hrs per night on average) were most of the excess sleep were reported the first night (12.5 h on average ranging from 10.5 to 13.5 h)

Discussion

Why do tau and Aβ increase?

The above results are indeed unexpected. Our initial expectation were to see a rise in biomarkers after the sleep-deprivation period followed by a fall in CSF concentrations after recovery. There are many possible explanations for the dramatic rise in amyloid beta and tau following recovery sleep, but first one needs to rise the question whether the high leves of Aβ and Tau actually arise from the sleep deprivation itself and that there is a lag between the CNS insult and the rise biomarkers.

There is also some reason to believe that Aβ and Tau might accumulate in neurons while subjects are sleep deprived because glymphatic clearance is hindered (6). When the test subjects later gets sleep in abundance, previously pooled peptides are excreted from the neurons and into the interstitial space fluid (ISF). Aβ and Tau then continue from the ISF and into the cerebrospinal fluid. This would support the previous findings by Nedergaard et al (6).

Another possible explanation would be an increase in synaptic activity, which is known to increase CSF levels of Aβ. Holtzman et al estimates that 70% of ISF amyloid beta is transmited to the ISF by synaptic endocytois (49, 50). They also suggest that this is done in an activity-dependent manner.

One finding that might support the idea that the increased Aβ levels are dependent on synaptic activity and regeneration is the big difference between methods. The two methods differ in what antibodies are used. The INNOTEST® β-AMYLOID immunoassay detects complete strains of Aβ in a precise way, but the not yet fully validated amyloid-β peptide Triplex Assay is also able to detect Aβ that has been truncated in a faulty way. One could speculate that the percentage of faulty Aβ will increase if the process of splitting amyloid precursor protein is forced to speed up.

Moreover, there is an interesting link between mammal hibernation/torpor-states and elevated concentrations of highly phosphorylated tau in CSF (51). The exact mechanism is not well understood and is specific to phospho tau but never the less is an interesting connection between our findings and prolonged sleep like states in animals. It should be noted that hibernation/torpor state does not mean sleep (52). During torpor state mammals experience sleep as a separate part of torpor.

Methodological weaknesses

So far this study has far to few data points to draw definite conclusions even though the results are convincing. As mentioned earlier, the results are not what we expected. Because of this our design do not monitor the recovery period quite as rigorously as one would want. So far we only have self reported data from this period. This is something that will be addressed in upcoming experiment rounds. Among other things, we will ad actigraphic surveillance in the recovery period. One could also question if there should have been a baseline period before the periods of sleep under surveillance though the effect to CSF chemistry were probably minimal.

Because of logistical reasons PSG data has not yet been analysed. This does not have any major impact on the findings so far as sleep data, at this time, is only used to make sure that protocol was upheld. As mentioned earlier, under materials and methods, the actigraph is precise enough to do this task.

An unfortunate effect of polysomnography and sleep labs is that they pose a risk of influencing the sleep of test subjects. The magnitude of this influence differs from small (usually) to profound (seldom). A classic phenomenon where the monitoring might have a considerable impact is the so called "First night effect", meaning that the test subject get less quality and quantity of sleep when he or she is not yet used to the setting. Test subject D experienced this phenomenon, as her last night of controlled normal sleep was also her first night at the sleep centre. In response to

this we decided to introduce a habituation night at the centre in the beginning of the controlled normal sleep period. This will decrease the risk of first night effect in the upcoming rounds of our experiment.

Among the first four test subjects, three experienced post dural puncture headache (PDPH). This is more than expected but at this time we cannot say much. So far there seem to be a tendency to have more PDPH when sleep deprived. There is evidence that sleep or time of day might effect the amount of PDPH (53). Young age also seems to be a risk factor apart from those factors that can be mitigated (as mentioned under Ethics). A point of caution is the fact that the one subject who did not experience headache is also the subject with less pronounced increase in CSF biomarker levels. This is something that needs to be considered when doing the final analysis of all test subjects.

Conclusions

Recovery sleep following a prolonged period of sleep deprivation induces elevated levels in CSF T-tau, P-tau and Aβ42. These markers are traditionally associated with brain neuropathological changes in AD, but their role in normal physiology is currently largely unknown. Cell and animal data suggest that tau and Aβ are secreted from neurons in an activity-dependent manner. There are also data indicating that they may reflect synaptic and neuroaxonal plasticity. Finally, both tau and Aβ expression are up regulated in mammals during hibernation. Perhaps this, along with sleep-associated changes in CSF dynamics, may explain the results seen so far.

These findings have grown our knowledge of known biomarkers and their correlation with sleep deprivation. We have also gained increased understanding in the short and long term effects of sleep deprivation on neurocognitive function and its contribution to the development of neurodegenerative diseases such as Alzheimer's disease. Our results also prompts many new areas of further research.

Populärvetenskaplig sammanfattning

Sömnbrist och den mänskliga hjärnan - finns svar i ryggmärgsvätskan?

Sömn är ett förvånansvärt lite utforskat område. Människan har länge vetat att god sömn är nödvändigt för god hälsa, samtidigt har vi fram till nyligen haft mycket vaga föreställningar om varför det är så. Ny forskning har dock börjat skrapa på ytan till de grundläggande fysiologiska mekanismerna som gör att vi måste sova. 2013 visade en forskargrupp för första gången hur skadliga ämnen förs ut ur hjärnans celler under sömn. Detta hade innan varit en teori som nu kunde bevisas genom ett sinnrikt experiment på möss. Det ämne man såg föras ut ur hjärnan kopplar vi idag samman med demenssjukdomen Alzheimer.

I vår pågående studie tittar vi på vad som händer med den vätska som omgärdar hjärnan och ryggmärgen och som kallas ryggmärgsvätska. Det är i den vätska de ämnen som förs ut ur hjärncellerna under sömn hamnar. Vi låter friska människor med normala sömnvanor utstå perioder med normal sömn och begränsad sömn. Efter varje period, som varar i fem dagar, tar vi prov på ovan nämnda vätska genom ett stick i ländryggen. Vi tar även prov tre dagar efter perioden med begränsad sömn. De ämnen som vi mäter har på olika vis kopplats samman med stress och skada på olika typer av celler i hjärnan.

Vad vi har sett så här långt är att halten av ämnen som fram för allt ökar vi Alzheimers sjukdom stigit dramatiskt tre dagar efter perioden med mindre sömn. Faktum är att värdena nått nivåer som annars bara ses vid just Alzheimers sjukdom. En möjlig förklaring kan vara att transporten av dessa ämnen ut ur hjärnans celler varit begränsad då deltagarna inte sovit så mycket som krävs. När de sedan får sova "ikapp" så har dessa ämnen forsat ut ur cellerna och in i ryggmärgsvätskan. En annan möjlig eller bidragande förklaring är att omsättningen av nervcellernas kopplingar till varandra, synapserna, ökar under sömn. Denna omsättning har visat medföra ökade halter av vissa av de ämnen som även stigit i vårt experiment. Det är även känt att många som

drabbas av Alzheimers sjukdom har sömnstörningar som förvärras under sjukdomens förlopp.

Magnituden av våra resultat förvånar även oss. De ger stöd åt tidigare forskning samtidigt som de skapar nya frågeställningar. Upptäckterna bidrar även med kunskap om mekanismer i människan som tidigare bara har studerats i djur. Vi hoppas att detta kommer bidra med ökad förståelse om de grundläggande mekanismer som utgör sömn. Resultaten bidrar även till mer kunskap om Alzheimers sjukdom vilket är ett tillstånd som innebär ett stort lidande för drabbade och anhöriga samtidigt som kostnaderna för samhället är enorma och ökande.

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