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A Functional Magnetic Resonance Imaging Study on Global Signal Variability After One Night of Total sleep Deprivation

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Funktionell magnetkameraundersökning av den globala signalens variabilitet i vila efter en natt av total sömnbrist

Bakgrund: Effekter av sömnbrist på hjärnan kan studeras med funktionell magnetresonanstomografi (fMRI). En parameter som kan förväntas att förändras av sömnbrist är den globala signalen: tidserien av den genomsnittliga hjärnaktiviteten. Den användas som kovariat i fMRI-studier för att eliminera effekten av huvudrörelser, andning och variabilitet i hjärfrekvens. Efter sömnbrist, globala signalens standarddeviation: "Globala signalens variabilitet" (GSV) ökar, sannolikt på grund av högre variation i vakenhet. Tidigare forskning på sömnbrist och GSV har inte tillräckligt tagit hänsyn till sömnighet, ögonens öppenhet, och tid i fMRI som också påverkar vakenhet. *Syfte:* Syftet var att undersöka effekter av total sömnbrist på GSV. *Material och Metoder:* Denna var en överkorsad studie med 38 försökspersoner. Försökspersoner genomgick fMR efter en natt av total sömnbrist och en natt av vanlig sömn. Under fMRI undersökning hade försökspersoner ögonen öppna och stängda. Deltagarna skattade deras sömnighet på Karolinska sleepiness scale (KSS). *Resultat:* GSV var högre efter total sömnbrist, än efter vanlig sömn (0.255 ± 0.020 , $p < 0.001$). GSV ökade med tid i MR-kameran (0.003 ± 0.001 , $p = 0.026$), och minskade när ögonen var öppna (-0.135 ± 0.033 , $p < 0.001$). När KSS inkluderades i modellen ökade sömnighet GSV (0.057 ± 0.009 , $p < 0.001$). *Slutsats:* Resultatet visade att sömnbrist höjer GSV. Detta visar att GSV beror på hjärnans funktionella tillstånd, och inte bara är en teknisk artefakt. Sannolik mekanism för ökningen i GSV är högre variabilitet i vakenhet. Ökning med tid och blundandet stödjer detta. Variabilitet i vakenhet som mekanism för GSV förändringar måste forskas vidare i framtida studier.

A functional magnetic resonance imaging study on global signal variability after one night of total sleep deprivation

Introduction: Sleep deprivation's effects on the brain can be studied using functional magnetic resonance imaging (fMRI). One parameter increases with sleep deprivation is global signal: the time series of average fMRI signal. Global signal is often used as a covariate in fMRI studies, as a way to eliminate noise, such as head motions, breathing and heart rate changes. After sleep deprivation, global signal's standard deviation: "global signal variability" (GSV) increases, most likely due to increased variation in wakefulness. Earlier research on sleep deprivation and GSV has not sufficiently accounted for sleepiness, eyes openness, and time in the fMRI, which also affect wakefulness. *Aims:* The aim was to investigate effects of total sleep deprivation on GSV. *Material and Methods:* This was a crossover study with 38 participants. Participants went through fMRI after total sleep deprivation and habitual sleep. During scanning, participants had their eyes open, and closed. Participants estimated their sleepiness using Karolinska sleepiness scale (KSS). *Results:* Total sleep deprivation increased GSV (0.255 ± 0.020 , $p < 0.001$). GSV increased with time in fMRI (0.003 ± 0.001 , $p = 0.026$), and lowered when eyes were open (-0.135 ± 0.033 , $p < 0.001$). Sleepiness increased GSV (0.057 ± 0.009 , $p < 0.001$). *Conclusions:* Results show that sleep deprivation, time and eyes being closed increase GSV. This shows that GSV is not simply noise, but depends on the state of the brain. Likely mechanism for GSV increase is higher wake-state instability. Increase caused by time and eyes being closed supports this notion, but it should be further investigated in future studies.

Keywords: "sleep deprivation", "resting state", "global signal", "global signal variability"

Abbreviations

DMN – Default mode network

ECG – Electrocardiography

EEG – Electroencephalography

EOG – Electrooculography

FC – Functional connectivity

fMRI – Functional magnetic resonance imaging

GS – Global signal

GSV – Global signal variability

IC – Intrinsic connectivity

ICN – Intrinsic connectivity network

KSS – Karolinska sleepiness scale

Introduction

Sleep deprivation

Different theories have been proposed to explain the purpose of sleep. These include saving energy during the night (1), clearing waste from the brain's interstitial spaces (2, 3), helping to form memories and learn (4), and reduce brain metabolism (5). Regardless of the purpose, sufficient sleep – according to the American Academy of Sleep Medicine and Sleep Research Society – is at least 7 hours per night on a regular basis (6). In Sweden, as much as 55% of adolescents do not achieve 8 hours of sleep per night (7), as is recommended for them (8). According to a US study on self-reported sleep deprivation, the percentage of people who sleep less than 6 hours per night has increased from 22.3% in 1985 to 29.2% in 2012 (9). However, such a trend was not found in a meta-analysis of 168 studies, from 1960 to 2013 (10). This may be explained by different trends taking place in different countries. There are many overlapping causes of sleep deprivation, including stress, anxiety, time spent in front of screens, recreational drug use, work schedules, having small children, jet lag, and a number of medical conditions, such as sleep apnea, depression, and hyperthyroidism (11, 12).

Effects of sleep deprivation

Short-term sleep deprivation disrupts memory consolidation (4), as well as attention, as it makes one prone to microsleeps, which are moments of sleep that last a few seconds (13). In addition, sleep deprivation increases irritability (14), decreases empathy (15), and reduces cognitive abilities (16). Sleep deprivation also impairs decision-making and increases risk-taking (15). In addition to short term effects, sleep deprivation also has severe long-term effects on the individual level. Sleep deprivation is a risk marker for at least Alzheimer's disease (17), heart disease (18), and diabetes mellitus (19).

In addition to health issues, sleep deprivation causes dangers in everyday life. Based on data from a sample of in-depth crash investigations conducted by the U.S. Department of Transportation, Tefft (20) estimated that 21% of fatal traffic accidents in the United States involve a sleepy driver. In health care, the sleep deprived workers pose a significant risk to patient safety (21). In addition to the human cost and cost to wellbeing sleep deprivation also has great economic costs. It has been estimated that insufficient sleep costs the United States from 280 to 411 billion dollars annually (22).

Many of the adverse effects of short-term sleep deprivation are mediated by sleepiness. Sleepiness is the tendency to fall asleep with an accompanying subjective feeling of sleepiness. Subjective sleepiness has been found to be an indicator of insufficient sleep and impaired waking function (23). It is important to gain knowledge on the effects of sleep deprivation and sleepiness on the brain.

Functional magnetic resonance imaging

One way to study the effects of sleep deprivation and sleepiness on the brain is functional magnetic resonance imaging (fMRI). fMRI uses blood oxygen level-dependent (BOLD) signal to detect which areas are active in the brain (24), as areas with more neuronal activity have a higher blood flow. The fMRI thus shows activity with a short time delay. As higher blood flow is not perfectly correlated with activity, fMRI is an imperfect way to measure neural activity. However, compared to other modalities, fMRI offers greater spatial specificity, and observed signal is in most cases significant of neural metabolism (25).

fMRI can be used to show synchronous activity between spatially separated areas of the brain. This synchronous activity is called functional connectivity (FC) (24). FC can be used to study brain activity during specific tasks, or it can be studied in resting state fMRI, which means that the person is asked to look at a fixation cross, or have their eyes closed, while doing nothing in particular. When studied in the resting state, FC is called intrinsic connectivity (IC). IC shows different networks of activity, called intrinsic connectivity networks (ICN). These networks are formed of specific, functionally connected areas that are active during resting state (26). These ICNs include default mode network, sensorimotor network, executive control network, visual networks, lateralized fronto-parietal networks, auditory network and temporo-parietal network (24). Of these networks, the one that's activity is most correlated to the resting state is the "default mode network". The default mode network is related to internally focused tasks including autobiographical memory retrieval, envisioning the future, and conceiving the perspectives of others (27). The default mode network is found to be affected in pathologies, such as Alzheimer's, Parkinson's, autism and Schizophrenia (26). Much of resting state fMRI research is focused on the default mode network.

Global signal

In the analysis of fMRI data, time series of fMRI signal intensity, averaged across the whole brain is known as the global signal (28). Global signal is – in part – caused by heart rate changes, head movement, breathing, and instability in the measurement (29). Global signal is often used as covariate when analysing fMRI data, in order to remove the effect of heart rate changes, movement, breathing and measurement instability (30). This way to use the global signal in resting state fMRI analysis is called global signal regression. One benefit of global signal regression is that it improves spatial specificity when mapping functional connectivity (31). On the other hand, global signal regression can introduce “artefactual” anti-correlations that were not originally present in the modelled data (28). The case against global signal regression is also furthered by results by Schölvinck et al. (29), which showed that a portion of the global signal is directly linked to neural activity. They were able to find a correlation between global signal changes, and local field potentials in the brain, by studying anesthetized monkeys. This result has awakened interest into the further role of global signal as a measurement that reflects actual activity in the brain, and not just confounding factors. To help resting state fMRI research, which has to grapple with the question of global signal regression’s usefulness, Chen et al. (32) have suggested a method to determine the necessity of global signal regression, which is based on quantifying the level of non-neural noise that has a global effect on the fMRI signal. Because global signal is caused both by neural activity and other factors, and global signal regression is used widely in intrinsic connectivity studies, it is important to better understand what underlies the global signal and how it is affected by participants’ activities.

Global signal variability

One way to analyse the global signal is to focus on global signal variability (GSV), which is the standard deviation of the global signal, as used by Wong et al. (31), though they called it the amplitude of global signal. Other sources also refer to GSV as global signal fluctuations (33). Wong et al. (31) showed, that caffeine intake decreases GSV. Conversely, Midazolam – a benzodiazepine – has been shown to increase GSV (34). Wong et al. (35) also showed that GSV is increased with changes in electroencephalography (EEG) measures, that are associated with vigilance. Based on this, Wong et al. (35) even suggested, that GSV might serve as a useful indicator of vigilance. A suggested mechanism for the increase of GSV with lower vigilance, is that onset of sleep affects the variation in respiration and heart rate, which in turn affect the global signal (36).

GSV and sleep deprivation

Partial sleep deprivation's effect on GSV has been studied in Stockholm sleepy brain project 1 (SSBP1), which was a cross-over study that compared resting state fMRI after a night of partial sleep deprivation with resting state fMRI after a night of habitual sleep (36). It was shown in SSBP1, that GSV is increased by partial sleep deprivation (36). This was to be expected as GSV increases with lower vigilance, and sleep deprivation increases participants' tendency to drift in and out of sleep during resting state fMRI. The tendency to drift in and out of sleep is known as wake-state instability. It was also shown in SSBP 1 that the increase in GSV – after sleep deprivation – is lower for older individuals, but GSV without sleep deprivation is higher. In addition to these effects on GSV, GSV is also affected by the circadian rhythm such that it decreases as the day progresses, as shown by Orban et al (33). This is not to be expected if GSV was simply correlated with sleepiness, and it demonstrates that there is more to know about GSV.

fMRI studies and sleep deprivation

It has been shown by Tagliazucchi et al. (37) that a third of participants in resting state studies - without sleep deprivation - fall asleep within 3 minutes. That study was conducted with the simultaneous use of EEG during the resting state fMRI. Based on that finding, it is reasonable to assume that sleep deprivation can have some unknown effects on resting state studies. One specific mechanism is that sleep deprivation would increase wake-state instability. Despite this, much of resting state fMRI research does not include wakefulness monitoring. As wakefulness is often not monitored in fMRI research, and sleep deprivation is expected to increase wake-state instability, it is important to gain a better understanding in what exact effects sleep deprivation can have on the brain in resting state, for the sake of future resting state fMRI research.

One of the effects of sleep deprivation is that GSV increases, possibly due to higher wake-state instability. However, earlier research on sleep deprivation and GSV has not sufficiently taken into account other variables that can – especially in combination with sleep deprivation – affect wake-state instability; specifically, subjective sleepiness, time in the resting state fMRI, and eyes being open or closed. These variables are important to include in order to further improve the knowledge on the interplay between sleep deprivation, wake-state instability and GSV.

Aim

The aim in this study was to investigate the effect of total sleep deprivation on global signal variability in resting state fMRI, together with the effects of subjective sleepiness, time in resting state fMRI and eyes being open or closed. Specific hypothesis was that sleep deprivation and sleepiness would increase global signal variability.

Materials and Methods

Study design

This study was cross-over study with 38 participants; 29 from main study, and 9 from an earlier pilot. It was a part of Stockholm sleepy brain project 2 (SSBP2). The participants went through MRI three times, the first time to take anatomical images of their brain. The second and third sessions were either after one night of sleep deprivation or after a night of habitual sleep. The order in which the participants underwent the sleep deprivation and the habitual sleep was randomized. Sleep deprivation took place at a sleep laboratory (Stockholm University) and habitual sleep took place at the participant's home. For the 9 participants in the pilot study, even sleep deprivation took place at home, with an actigraph for monitoring. After the night of sleep deprivation, the participants were transported with taxi to Karolinska University Hospital for experiments at 7 am. The scans were performed between 8 am and 10 am. For most mornings, there were 2 participants, who had both undergone the same protocol of either sleep deprivation or habitual sleep. On some mornings there was only 1 participant. One participant began scanning at 8 am, and the other at 9 am. When participants underwent habitual sleep, they were expected to be at the sleep laboratory at 7.30 am. or 8.30 am. The participants were not allowed to consume any caffeine in the 24 hours preceding the scanning.

Resting state data was gathered by doing an fMRI scan while the participant first looked at a fixation cross for 5 minutes. During this time, the participants' eyes were monitored with an eye tracker, and a wakeup call was sounded if they close their eyes for more than 5 seconds. Then the participant was allowed to close their eyes for 15 minutes, while the scanning continued. Participants' respiratory frequency, heart rate and electrocardiography (ECG) were monitored as well.

Other types of data were also gathered in SSBP2. When the participant went through resting state scanning, they had already been in the fMRI scanner for approximately 40-50 minutes, during which their empathy for pain and reaction speed were tested. In total a participant spent roughly an hour in the scan per session. After the resting state fMRI blood was gathered for analysis and the participant performed a stroop test, a test of delay in reaction time between congruent and incongruent stimuli (38).

The participants estimated their sleepiness using the Karolinska sleepiness scale (KSS), a 9-point scale, where 1 = extremely alert, 3 = alert, 5 = neither alert nor sleepy, 7 = sleepy – but no difficulty remaining awake, and 9 = extremely sleepy – fighting sleep (23, 39). The participants estimated their sleepiness a total of 4 times per session. The last estimation – done immediately after the resting state scan – was used for analysis of the effect of KSS on GSV.

Participants

Participants were recruited through posters in campus areas and a student affiliated website (studentkaninen.se). Before the first MRI scan, the participants had to complete questionnaires to determine their suitability for this study.

Participants were required to be 20-30 years old (main study), or 18-65 years old (pilot study). They had to be right-handed to use a response box, designed for use with the right hand in another part of the study. They had to also be fluent in Swedish, live in Stockholm area and have normal eyesight. They also had to have a usual bedtime between 22.00-01.00.

Participants with metal objects in their body or claustrophobia were excluded, because of dangers in MRI. Participants with depression or insomnia were excluded. Depression and insomnia were assessed with Hospital Anxiety and Depression Scale (HADS-D) (40) and Insomnia severity index (ISI) (41) respectively. Specifically, $(HADS-D) \geq 8$ and $(ISI) \geq 15$ were the exclusion criteria. Participants with caffeine consumption in excess of 4 cups of coffee per day were excluded to avoid withdrawal symptoms. Habitual nicotine or psychoactive medication use were excluded because these substances disturb sleep (42-44). Participants with diabetes, hypertonia, earlier heart surgery, sleep apnea or immunomodulating drug use were excluded. Pregnant women were excluded. Additionally, students studying medicine, nursing or psychology, and those working in these fields were excluded because of a separate part of

the study focusing on empathy, that would be affected by participants in the medical field.

In total, 38 participants completed at least one of the fMRI sessions, and their data was included in the study. This included 9 participants from an earlier pilot study, and 29 participants from the SSBP2 proper (Fig. 1). Of the 38 participants, 34 participants completed both resting state fMRI sessions. 3 participants completed only the habitual sleep fMRI session, and 1 participant completed only the sleep deprived fMRI session.

Figure 1. Flowchart

Participant characteristics are shown in Table 1. Of the 38 participants included in data analyses, 15 were female, and 23 male. Because the 9 participants in the pilot study had different age criteria, 3 of them were over 30 years old. Even in the main study, two participants were at least

30 years old, and one under 20, despite exclusion criteria. However, none of the participants were over 35, so these issues are not determined to significantly affect the results.

Table 1. General characteristics of participants

	<i>mean</i>	<i>Standard deviation</i>
<i>age</i>	24.8	4.2
<i>HADS-D</i>	1.9	1.4
<i>ISI</i>	5.4	4.1

HADS-D = Hospital Anxiety and Depression Scale – Depression, ISI= Insomnia Severity Index

Data acquisition

A 3T GE 750 MRI scanner was used to acquire images. T1-weighted structural baseline images were acquired in the axial plane with 180 slices and used as anatomical reference. fMRI image acquisition was interleaved starting at the bottom, 40 slices, 3 mm thickness, and 0,5 mm spacing. There were 2 seconds between each volume.

Type of data

MRI images and KSS estimations were collected in this study. The data was pre-processed using a standard pipeline on fMRIPrep. Pre-processing included slice timing, realignment, normalisation and smoothing.

Quality control of MRI images

After pre-processing, MRI images were quality controlled manually with MRIQC, to make sure the whole brain is seen in the images (See Fig. 2). fMRI summary plots were manually inspected (See Fig. 3).

Figure 2. An example of fMRI images. Anatomical reference structures marked in purple and blue

Figure 3. Example fMRI summary plot. GS – global signal, GSCSF – global signal within cerebrospinal fluid, GSWM – global signal within white matter, DVARS – derivative of root mean squared, RMS – variance over voxels, FD – framewise displacement. Carpet plot shows a summary of

BOLD signals. Blue: cortical, orange: subcortical, green: cerebellar, red: white matter & cerebrospinal fluid.

Statistical analyses

GSV was calculated by dividing the global signal time series into 30 second blocks and calculating the standard deviation of the global signal in each block. This standard deviation of each block was then log transformed, to account for the data being skewed. All figures for GSV in this study are presented log transformed.

GSV was analysed using mixed effects models. In these models, participant identity was considered as a random effect. The different fixed effects in these models were condition: sleep deprived or habitual sleep, eyes open/closed, KSS evaluation and time spent in the resting state fMRI, which is called time on task. Time on task was analysed by comparing the aforementioned 30 second blocks.

In total, 6 different mixed effects models were used to analyse the effects on GSV. Model 1 included condition, time on task, and eyes open/closed as fixed effects. Model 1 excluded KSS, to capture the effect of sleep deprivation, without confounding with sleepiness. Model 2 included condition, KSS, time on task and eyes open/closed as fixed effects. Models 3 and 4 included condition, KSS and time on task as fixed effects. In these models, the data were stratified by eyes open/closed such that model 3 had data with eyes open and model 4 had data with eyes closed. This stratification was done because eyes openness and time on task confound with each other. Models 5 and 6 included KSS, time on task and eyes open/closed as fixed effects. In these models, the data were stratified such that model 5 had data from condition habitual sleep and model 6 had data from condition sleep deprived. This was done to analyse the effect of sleepiness, independent of sleep deprivation. The data was analysed using R with nmle package. Significance level was set to $p < 0.05$. All results are presented as mean $\pm$ standard error and p-value.

Ethical considerations

All participants provided informed consent before partaking in the study. They were provided with written information regarding the study. All data was anonymized. Possible harm to participants could have been claustrophobic reaction in the MRI, and side effects related to sleep deprivation. Claustrophobia was an exclusion criterion, and any side effects of sleep

deprivation after one night are most likely reversed after habitual sleep. Participants were offered a taxi ride to avoid traffic accidents. The participants were given 1500 SEK for participation. The biggest risk of harm entailed for the participant by these experiments was that something abnormal would have been found in the MRI scan. A radiologist examined the images, and any abnormal finds were investigated appropriately. The images were also linked into the participants' medical journals for their own benefit. No pathological findings were made. An incidental finding could have helped the patient, in case something that demanded treatment was found, but it might have caused unnecessary stress to the participant. It is important to gain a deeper understanding of the effects sleep deprivation on the function of the brain. Possible harm caused to the participants was considered small. Therefore, the benefits outweigh the risks. The study was done in accordance with the Helsinki declaration. Ethical permit was required and exists. Ethical review number 2012/1098-31/2

Results

Effect of sleep deprivation on sleepiness

As expected, the participants gave a higher KSS rating after one night of sleep deprivation (2.9 ± 0.4 , $p < 0.01$). (See Fig. 4)

Figure 4. Karolinska sleepiness scale (KSS) ratings. Participants gave a higher KSS rating after one night of sleep deprivation (2.9 ± 0.4 , $p < 0.01$).

Effects of sleep deprivation, sleepiness, time on task and eyes' openness on global signal variability

GSV in resting state fMRI after habitual sleep and sleep deprivation is shown in Figure 5. Results from all 6 mixed effects models are shown in Table 2. GSV was higher after total sleep deprivation than after habitual sleep (0.255 ± 0.020 , $p < 0.001$), in model 1. When the effect of KSS was included in model 2, GSV was significantly increased by KSS (0.057 ± 0.009 , $p < 0.001$).

Figure 5. Global signal variability (GSV) under resting state fMRI. GSV is shown log-transformed. The red line is the mean GSV after total sleep deprivation. The black line is the mean GSV after habitual sleep. 5 minute line to show when the participants were allowed to close their eyes. Longer lines represent participants from the pilot study. $N=38$. GSV was higher after total sleep deprivation than after habitual sleep (0.255 ± 0.020 , $p<0.001$). GSV was significantly lower when the participants had their eyes open (-0.135 ± 0.033 , $p<0.001$). GSV was significantly increased by time on task; there was a significant increase with each additional 30 s block (0.003 ± 0.001 , $p=0.026$).

In model 1, GSV was significantly lower when the participants had their eyes open (-0.135 ± 0.033 , $p<0.001$). In the same model 1, GSV was significantly increased by time on task; there was a significant increase with each additional 30 s block (0.003 ± 0.001 , $p=0.026$). Data was stratified by eyes open/closed for models 3 and 4, and condition, KSS and time on task were fixed effects. This was done to analyse the effect of time on task independent of eyes open/closed. GSV was significantly increased by time on task, when eyes were open (0.036 ± 0.006 , $p<0.001$), and GSV was not significantly increased by time spent on task when eyes were closed (0.001 ± 0.001 , $p=0.396$).

Data was also stratified by condition habitual sleep and sleep deprivation, and analysed in models 5 and 6. These models included KSS, time on task, and eyes open or closed as fixed effects, in order to analyse the effect of KSS specifically, independent of condition. KSS had

no significant effect in either the habitual sleep condition (0.027 ± 0.029 , $p=0.369$), or the sleep deprived condition (0.023 ± 0.076 , $p=0.767$).

Table 2. All results on the effects on GSV. Model 1 included condition, time and eyes open/closed as fixed effects. Model 2 included condition, KSS, time and eyes open/closed as fixed effects. Models 3 and 4 included condition, KSS, time and eyes as fixed effects. They were stratified by eyes open/closed such that model 3 had data from eyes open and model 4 had data from eyes closed. Models 5 and 6 included KSS, time and eyes open/closed as fixed effects. They were stratified such that model 5 had data from condition habitual sleep and model 6 had data from sleep deprivation. All data reported as median $\pm$ standard error, p-value.

	Condition	KSS	Time	Eyes open
Model 1	0.255 ± 0.020 , $p<0.001$	–	0.003 ± 0.001 , $p=0.026$	-0.135 ± 0.033 , $p<0.001$
Model 2	0.090 ± 0.032 , $p=0.005$	0.057 ± 0.009 , $p<0.001$	0.002 ± 0.001 , $p=0.064$	-0.124 ± 0.034 , $p<0.001$
Model 3	0.178 ± 0.053 , $p<0.001$	0.046 ± 0.015 , $p=0.002$	0.036 ± 0.006 , $p<0.001$	–
Model 4	0.065 ± 0.038 , $p=0.087$	0.059 ± 0.011 , $p<0.001$	0.001 ± 0.001 , $p=0.396$	–
Model 5	–	0.027 ± 0.029 , $p=0.369$	0.006 ± 0.002 , $p<0.001$	-0.093 ± 0.043 , $p=0.029$
Model 6	–	0.023 ± 0.076 , $p=0.767$	-0.002 ± 0.002 , $p=0.212$	-0.163 ± 0.048 , $p<0.001$

KSS = Karolinska Sleepiness scale

Discussion

Summary

GSV was analysed in resting state fMRI with participants who were either sleep deprived for 24 hours or had had a habitual sleep prior to the fMRI sessions. GSV is the variability of the global signal in fMRI imaging. The results show that total sleep deprivation increases GSV. GSV was also increased by eyes being closed, and time spent in the fMRI. The effect of subjective sleepiness, rated with KSS increased GSV, but this effect became nonsignificant, when data was stratified by condition habitual sleep and sleep deprived.

Interpretation

The results of the effect of sleep deprivation on GSV are in concordance with earlier studies on the matter. Increased GSV after partial sleep deprivation has earlier been reported in SSBP1 (36). The same effect has also been reported by Yeo et al. (45).

Wake-state instability

A likely mechanism for the observed increase in GSV after sleep deprivation is wake-state instability, an increased tendency to fall asleep. High wake-state instability has been observed by Tagliazucchi et al. (37) during resting state fMRI. Wake-state instability may increase GSV by changing respiration and pulse, which affect GSV, or it may change metabolic requirements of nerve cells in the brain when switching between wake and sleep (36).

That wake-state instability would be behind GSV's increase is supported by the finding, that GSV was not only increased by sleep deprivation, but also by time on task and eyes being closed, all of which can reasonably be expected to make falling asleep more likely. Time on task was only statistically significant when the participants were supposed to keep their eyes open. This can be explained by successively increasing wake-state instability during this period, and the participant then falling fully asleep afterwards, when they had closed their eyes. In effect, GSV increased to a higher level and roughly held that level after eyes were closed. Another explanation for this phenomenon could be, that the successive increase in GSV happened because they were the first few minutes of the resting state scan. This explanation is somewhat contradicted by the fact, that participants had already been in the scanner for 40-50 minutes, doing other tasks. Thus, they were already used to being in the machine, and the observed rise in GSV cannot be explained, for example, by some initial nervousness – caused by the scanning – fading away gradually.

Sleepiness

The increasing effect of subjective sleepiness on GSV, as estimated with KSS became nonsignificant, when the data was stratified by condition habitual sleep and sleep deprived. This is somewhat surprising, because sleepiness is thought to be the mechanism that mediates the effects of sleep deprivation. Therefore, higher subjective sleepiness should be expected to increase GSV, even when it is not caused by supervised sleep deprivation. Earlier research has shown that caffeine decreases GSV, Midazolam increases GSV, and GSV is related to vigilance (31, 34, 35). Based on these results it is reasonable to expect that a high KSS rating per se, even when unrelated to supervised sleep deprivation, would predict a higher GSV.

What may explain the results on the effect subjective sleepiness on GSV is that KSS predicts higher GSV, only as far as the KSS estimate is related to sleep deprivation. That is to say, an increase in GSV is specifically related to sleep deprivation, more so than to the sleepiness caused by sleep deprivation. However, it could also be that the effect of subjective sleepiness

is too discreet to be captured in this study. This is somewhat contradicted by the wide variation in the KSS estimates in condition habitual sleep. Despite this, it is still possible, that this study was too small to capture the effect of subjective sleepiness on GSV. Another possibility is that the precarious nature of a subjective estimation, such as the KSS, is simply not very stable between different individuals and situations. This raises the question of whether asking study participants how many hours they have slept would be more predictive of parameters associated with sleep deprivation. However, KSS does predict GSV well when the condition (sleep deprived or habitual sleep) of the participants is not accounted for, and the model that included both KSS and subject condition showed both condition and sleepiness to have an effect on GSV. An interpretation of these results is that sleepiness does mediate some of the effect of sleep deprivation.

It is interesting to note that there was wide variation in the KSS estimates in the habitual sleep condition. Some reasons for this may be individual differences; that is, some people may be generally more tired in the morning, the early starting time for the scanning sessions may have forced some participants to wake up earlier than usual. The sessions also started at different times for different participants. These KSS estimates might have been different if the scanning had started later in the morning. In any case, despite the results on the effect of KSS on GSV, the variation in subjective sleepiness makes it plausible that participants' sleepiness can have unknown effects on results in fMRI studies. Because sleepiness and sleep deprivation are affected by the time of day in a specific study, and because GSV has been shown to decrease as the day progresses (33), it is important to consider the time of day when an fMRI-study is conducted.

The results of this study demonstrate only a fraction of the effects of sleep deprivation on resting state fMRI. Some of the effects of sleep deprivation are, as of yet, unknown. Thus, sleepiness or sleep deprivation may affect the results of other resting state fMRI studies in hitherto unknown ways. It follows, that it may be important to account for sleep deprivation and sleepiness in resting state fMRI studies.

Strengths

Strengths of this study include that participants were supervised during their night of sleep deprivation. Not monitoring the participants during the night of sleep deprivation would be a

source of error in that there would be uncertainty on whether they fell asleep during the night. Participants' eyes were monitored during the fMRI. Several earlier studies, with a similar design to this one have not included eye-monitoring. 38 participants is more than most similar studies have had. Using total sleep deprivation can be considered a strength, because it should cause greater effects than partial sleep deprivation.

Limitations

Using electroencephalography together with fMRI would be useful for better temporal specificity, but combining these methods is technically challenging. Electrooculography (EOG) was intended to use to monitor wakefulness, but a clear signal was not obtained, in addition one participant suffered a minor skin burn at an electrode site, so the use of EOG was abandoned. Heart rate, ECG and respiratory rate could not be analysed, despite intention to do so.

The study population consisted of participants under the age of thirty. Therefore, the results of this study are not representative of all ages, because global signal, and the effect of sleep deprivation on global signal differs for different ages. Total sleep deprivation has lower ecological validity than partial sleep deprivation because it does not occur in society as much as partial sleep deprivation.

Roughly half of the participants were expected to be at the sleep laboratory at 7.30 after their night of habitual sleep, and the rest at 8.30. Many participants were students, and students may differ from the general population, when it comes to sleep rhythm. This issue was managed by having ISI score >15 as an exclusion criterion, and habitual bedtime at 22.00-01.00 as an inclusion criterion, despite these measures taken, it is still possible that the starting time caused some participants to deviate considerably from their normal routine. This puts into question how representative the results of this study are of GSV after habitual sleep. However, the main aim of this study was not to describe GSV after habitual sleep, but to investigate the effect of sleep deprivation on GSV, and robust results were found. Therefore, possible change to the participants' habitual sleep schedule was not considered a problem. It may have influenced analyses of the effect of sleepiness, where some separate analyses were done on the two different conditions. Data collection was forced to discontinue 2020-03-18, due to the coronavirus pandemic. Therefore, data was not collected from 50 participants as intended.

Significance

The result, that GSV increases with sleep deprivation reinforces the idea, that global signal is not simply a source of noise in fMRI, but is related to the functional state of the brain. GSV's relationship to sleep and wakefulness is interesting for further study. It is likely that GSV is increased by higher wake-state instability, either because wake-state instability changes respiration and pulse, or wake-state instability changes metabolic requirements of nerve cells. In addition to this, these results further demonstrate the importance of wake-state monitoring and considering the effects of possible sleep deprivation in resting state fMRI studies.

Equity

This is a pre-clinical study and the results have no implications for clinical applications at this moment. The results of this study are not representative of all ages. As only healthy participants were included in this study, it is possible, that the results are not generalizable to those suffering from neurological diseases.

Future studies

Global signal variability after partial sleep deprivation was studied in younger and older age groups in SSBP1. GSV after total sleep deprivation in older groups has not been studied sufficiently. Based on the result of this study, that total sleep deprivation increases GSV and the result of the earlier SSBP1, that partial sleep deprivation increases GSV, it could be studied whether total sleep deprivation causes a higher increase in GSV than partial sleep deprivation. Based on the results with KSS, it may be of use to study the ability of KSS to predict GSV, compared to – for example – participants' estimates of how much they slept the preceding night. ECG, respiratory rate and EOG were attempted to use in this study, but a good measure of wake-state was not obtained. These should be used to monitor participants' wake-state in future studies, in order to properly capture the changes and effects of wake-state instability. EEG as well could be used in future study to better monitor vigilance.

Conclusions

Sleep deprivation increases subjective sleepiness. Sleep deprivation increases GSV. Time spent in the fMRI and closing of the eyes also increased GSV. This shows that GSV depends on the functional state of the brain, rather than being merely a technical artefact. The likely mechanism by which GSV increases due to sleep deprivation is wake-state instability, and the results of

this study are in concordance with that notion. However, further studies are needed to investigate it further.

Contributions

Our research group consisted of Gustav Nilsonne, Paolo d’Onofrio, Sandra Tamm, Torbjörn Åkerstedt, Mats Lekander, Heather Rusch and Jimmy Hermansson. Joe Wexler and Gustav Nilsonne did the MRI data preprocessing. Sanna Graniittiaho and I used data from the same set of participants in our degree projects. We both supervised participants during their sleep deprivation, participated in data collection, and analysed data.

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