Master Thesis

Neurocognitive Psychology (M. Sc.)

Functional Connectivity of Partial Sleep Deprivation in a Mature Group:

An rs-fMRI Study

Submitted by

Ana Paulina Lira García

Matriculation Number: 4398047

First Examiner: Dr. Peter Sörös

Second Examiner : Prof. Dr. Jochem Rieger

Oldenburg, 07.10.2019

Contents

List of Figures

List of Tables

List of Abbreviations

ANTs	Advanced Normalization Tools software
AROMA	Automatic Removal Of Motion Artifacts
BET	Brain Extraction Tool
BOLD	Blood-oxygen-level-dependent
DMN	Default Mode Network
EEG	Electroencephalography
FEAT	FMRI Expert Analysis Tool
FLIRT	FMRIB's Linear Image Registration Tool
FMRIB	Oxford Centre for Functional Magnetic Resonance Imaging of the Brain
FSL	FMRIB Software Library
FWHM	Full Width at Half Maximum
gICA	group Independent Component Analysis
GLM	General Linear Model
HADS	Hospital Anxiety – Depression Scale
IC	Independent Component
ICA	Independent Component Analysis
ISI	Insomnia Severity Index
KSQ	Karolinska Sleepiness Questionnaire
KSS	Karolinska Sleepiness Scale
MELODIC	Multivariate Exploratory Linear Optimized Decomposition into Independent Components
MNI	Montreal Neurological Institute
PSD	Partial Sleep Deprivation
rs- fMRI	resting-state functional Magnetic Resonance Imaging
SD	Standard Deviation
TR	Repetition Time

Abstract

Introduction. The main purpose of this thesis was to investigate the functional connectivity of partial sleep deprivation (PSD) in a group of healthy elderly subjects (60-75 years). A number of previous studies have investigated resting state activity in a younger age range. Here we wanted to replicate the premises of previous studies on sleep deprivation carried out in young groups and extend the research to the older population using resting state functional magnetic resonance imaging (rs- fMRI). Finding signaling spikes in spatial dimensions that suggests that those brain regions are passing information implies a connection. This study entails to analyze the spontaneous fluctuations of the signal, meaning, when there is no specific cognitive demand for the subject (Bijsterbosch, Smith & Beckmann, 2017).

Methods. The data was provided by the Stress Research Institute, part of the Faculty of Social Science from the Stockholm University, via OpenfMRI (https://www.openfmri.org/dataset/ds000201/). The data contained both anatomical and functional images taken at a rest state. Scanning was made after normal sleep and partial sleep deprivation of 3 hours on a crossover design (one month between sessions). We conducted a data analysis to investigate functional connectivity from 24 mature participants (14 females, 10 male). Independent component and subsequent network analysis were done using FSL. Motion correction, standard volume-realignment followed by independent component analysis-based automatic removal of motion artifacts (FSL's ICA-AROMA) were employed (Sörös et al., 2019).

Results. This thesis demonstrates the feasibility of producing canonical networks in a group level decomposition on resting state data. Based on the pertinent literature, this study expected to find a decreased connectivity within the default mode network (De Havas et al., 2012). On the contrary, it did not obtain any significant changes in the mentioned network. In contrast, it is worth noticing that this thesis found a decreased activity in the cerebellar network (Left VI), as originating from the partially sleep deprived condition.

Discussion. The performed analysis in this thesis was merely exploratory and fulfilled its investigative function satisfactorily. It addressed the fact that the ICA methodology approaches the underlying BOLD signals, substantiating the previous findings on resting state functionality. Consistent with existing literature, cerebellar activity is affected by lack of sleep. Malfunctions of the cerebellum are generally accompanied by sleep disorders and vice versa (Canto et al., 2017, Cunchillos & De Andrés,1982, DelRosso & Hoque, 2014). Nonetheless, elucidating about variations in the cerebellar network is futile if it is not accompanied by other variations in the cerebral cortex. Further research is needed to accurately establish the impact of sleep deprivation on the cerebellum and its cerebral connections.

1. Introduction

1.1. Sleep Deprivation

The first experimental study of cognitive impairment due to the absence of sleep was published in 1896 by the psychologists G. T. W. Patrick and J. Allen Gilbert. Thousands of articles have been published regarding the effects of total sleep deprivation, but only a small fraction approaches partial sleep deprivation, and an even smaller one is about the older population. Memory, emotion, mood, cognitive and motor performance, are some of the most popular measures used to research the effects of sleep deprivation (Chokroverty, 2017). The consequence of nearly all types of sleep deprivation results in an increase of negative states of mind and emotions, such as confusion, somnolence, decreased energy, lower motivation, and fatigue (Goel et al., 2009).

1.1.1. Effects of Sleep Deprivation

It is well known that a sleepless night can have negative impacts, specially in acute conditions. The certitude that sleep deprivation leads to a deterioration of cognitive performance is ubiquitous among the available literature on the deficit of sleep (Goel, Rao, Durmer & Dinges, 2009). Affected cognitive functions include vigilant and executive attention, psychomotor and cognitive speed and working memory, just to quote some higher cognitive skills (Tamm, 2019). In an exhaustive article about this health concern published by Namni Goel et al. in 2009, reports how cognitive deficits hoard over time, even unnoticed by the individual. As if the cognitive effects were not enough, lack of sleep is also associated with negative social effects such as high financial costs, both for the individual as well as for the health institution that supports them (Goel et al., 2009). Sleep problems are a public health concern. Being awake for more hours than biologically possible, a reduction in the quantity (and quality) of sleep, and prolonged driving duration, are all related to driving in a drowsiness and fatigue state, contributing to the occurrence of car accidents. Falling asleep while driving is particularly common, but often underestimated (ibid).

1.2. Aging Brain and Sleep Deprivation

There is not much previous literature that encompasses the analysis of sleep deprivation within a group of older people. Just as Nilsonne confirmed in his 2017 publication, only one prior study investigated the affinity between age, sleep deprivation and connectivity. Thereafter, Nilsonne himself and Tamm took the second chair. The first study I am referring to, suggest that sleep deprivation resembled the aging brain, thus their research was based on a comparison between sleep deprived young subjects and non-sleep deprived old subjects (Zhou, Wu, Yu, & Lei, 2017). This group stipulated that, if younger subjects did not sleep, the brain connectivity of some regions would decrease to such an extent that they would resemble the functional connectivity of the older group of subjects, which was not sleep deprived (ibid).

Aging is accompanied by certain changes in sleep patterns, such as sleep duration, sleep continuity (increased number of awakenings during the night), sleep intensity (decreased slow wave activity on EEG), lower arousal threshold that produces a decrease in the sleep depth, and an overall decline in quality and duration (Duffy, Willson, Wang & Czeisler, 2009). These changes occur even in the absence of clinical disorders (ibid). The older population has more sleep problems than the younger one; they have more discomfort caused by lack of sleep and the quality of their rest is significantly reduced (Tamm, 2019). These are important reasons why a mature group was chosen for analysis, including also the motivation to broaden knowledge in a field that, so far, has been scarcely researched. The Sleepy Brain Project results report that, compared to a younger group, somnolence is reduced in older people both when they are partially sleep deprived, as well as when they slept as usual (Nilsonne et al., 2017). Tamm's results indicate that the younger group appears to be more susceptible to lack of sleep (sustained through the physiological examination and the sleepiness questionnaires) (Tamm, 2019). In addition, sleep duration and the ability to produce sleep under optimal conditions is believed to decrease with age, suggesting that the need for sleep is reduced (Nilsonne et al., 2016). Due to possible structural changes in white matter that are part of the natural aging of the human brain, functional connectivity is generally lower compared to younger ones (Tamm, 2019).

1.3. The Resting State

1.3.1. Intrinsic Brain Functionality

In 1870 already, The US psychologist and philosopher William James managed to capture the complexity of what would become a dichotomous debate regarding brain function: *“Whilst part of what we perceive comes through our senses from the object before us, another part (and it may be the larger part) always comes out of our own head”* (James, 1870). One of the first researchers in the field of resting state studies, Marcus Raichle (2010), endorses two approaches to the functionality question: one posits that the brain works mostly due to external stimuli and, as a result of temporary requirements produced in the environment; the second stipulates that brain processes are mostly intrinsic, and supposes the acquisition and sustenance of information that allows one to interpret, respond and predict external events. A preponderance of fMRI studies has focused on task-related responses. These experiments tacitly foster a reflective comprehension of brain function. The approach is undoubtedly advantageous, however it also ignores the plausibility of intrinsic brain function (Raichle, 2010).

In Raichle's words, one of the most persuasive arguments about the importance of intrinsic activity arises from the consideration of its cost of brain energy consumption. Relative to the rate of energy consumption at rest, changing to an active state denotes a minimal increase in metabolic consumption, often no more than 5%. Fluctuations in brain activity rarely affect the overall rate of cerebral blood flow and metabolism, even when more strenuous activities are performed (Raichle, 2010).

1.3.2. Connectivity

Under the above predicate, the study of resting scans gained major attention. Nowadays it also seems intuitive to deduce that the brain networks are active even at rest. While Raichle worked on the breakdown of metabolic consumption, Stephen Smith, Christian Beckmann and colleagues concomitantly assessed the question of neuronal activity when the brain is conducting neither a cognitive nor a physical task. Studying spontaneous fluctuations, they deduced that brain networks show an analogous pattern between the analyses performed on the brain at rest and in activity (Bijsterbosch, Smith & Beckmann, 2017, Jenkinson, Beckmann, Behrens, Woolrich & Smith, 2012, Nilsonne et al., 2017). This group of pioneers working on the resting state opened very important paths when it comes to what we know today about the brain maps where upon not performing a specific task. There are two fundamental premises to emphasize, which have sustained the resting state approach in the last two decades: firstly, that the spontaneous blood oxygen level dependent (BOLD) fluctuations are not fortuitous artifacts, but rather are functionally correlated between brain regions and anatomical systems; and secondly, that the noise coming from the cardiac and respiratory activity is not responsible for the detected correlation patterns (Bijsterbosch, Smith & Beckmann, 2017, Jenkinson, Beckmann, Behrens, Woolrich & Smith, 2012, Nilsonne et al., 2017, Raichle, 2010, Sonuga-Barke & Castellanos, 2007).

Talking about connection refers to the communicative capacity of one area of the brain to communicate with another. Two or more regions located in different areas, but showing resemblances in their BOLD signals over time, are functionally connected

(Bijsterbosch et al., 2017, Fox et al., 2005, Raichle, 2010,). In order to investigate connectivity, it is necessary to measure the resemblance of the brain signals, i.e. to find signaling spikes in spatial as well as in temporal dimensions that suggests that those brain regions are passing information, implying a connection between the concerned areas. Functional connectivity is typically defined as "the observed temporal correlation (or other statistical dependencies) between two electro- or neurophysiological measurements from different parts of the brain" (Bijsterbosch, Smith & Beckmann, 2017, p. 3). The analysis of connectivity requires to study the spontaneous fluctuations of the signal, meaning, when there is no specific cognitive demand for the subject.

1.3.3. The Importance of Resting State Research

There are multiple reasons why it is important to study the brain at rest, since without a doubt this broadens our knowledge regarding its functionality. As mentioned above, the resting state is the recourse to study the inherent organization of the brain. It should not be neglected that a better understanding of the intrinsic infrastructure of the brain is a primary neurological concern, just as its underlying communicative levels (Fox et al., 2005, Raichle, 2010, Bijsterbosch, Smith & Beckmann, 2017). It enables us to discern different brain areas, and detect activity in established networks, allowing them to be evaluated and analyzed. This constancy is what makes it a completely reliable method (Beckmann, DeLuca, Devlin & Smith, 2010). As Bijsterbosch, Smith, and Beckmann (2017) have explained, this type of research could help us comprehend how the brain is capable of processing such complex information, such as behavior, thoughts and motivations. Knowing how the communication model works can clarify why things tend to fail in a whole spectrum of disorders. Another benefit is, by better understanding the brain in its basal state, the knowledge of how the brain 'activates' in the face of cognitive demands or tasks can be broadened further (Smith et al., 2009).

The vast majority, though not all, of current neuroimaging studies are based on the principle of cognitive subtraction. This concept refers to the comparison of two conditions which only differ in a single aspect: the independent variable (Harrison B.J., Pantelis C., 2010). This definition premises that a cognitive process can be added or inserted into a task without affecting the remaining processes, ignoring a possible interaction between them, which is called "pure insertion" (ibid). The interaction of cognitive processes is quite complex, so this assumption is probably violated in many situations. Even the simplest processes such as attention and working memory, are involved in everyday life (Fox et al., 2005). This cannot be ignored. Studying spontaneous fluctuations may eliminate the problem of "pure insertion", evaluating the possible influence of recent experiences or the current cognitive state of the subject (Bijsterbosch, Smith & Beckmann, 2017). Resting state also has a great potential to serve as the biomarker for mental disorders. A biomarker is something that can be measured accurately and can be reproduced, and therefore serve as an objective indication of the medical state of a person over time (ibid).

1.3.4. Resting State Networks

As defined in the English Oxford Dictionary, a group or system of interconnected people or things with a particular purpose is a network. Given the definition of functionality as described by Bijsterbosch and colleagues, a resting state network is a set of brain regions that exhibit affinity in their time series obtained during rest (Bijsterbosch, Smith & Beckmann, 2017). To date there still is no exact understanding of how the resting state networks operate, but we can obtain an insight on the basis of their predictable patterns (Beckmann, DeLuca, Devlin & Smith, 2005). One of the advantages of the RSN study, is that a number of networks have already been recognized, as they can always be observed in resting scans, and, in addition, are completely replicable (Smith et al., 2009). Figure 1 shows the networks identified by Smith and colleagues, who named them based on the parallelism to task-based regions (Bijsterbosch, Smith & Beckmann, 2017).

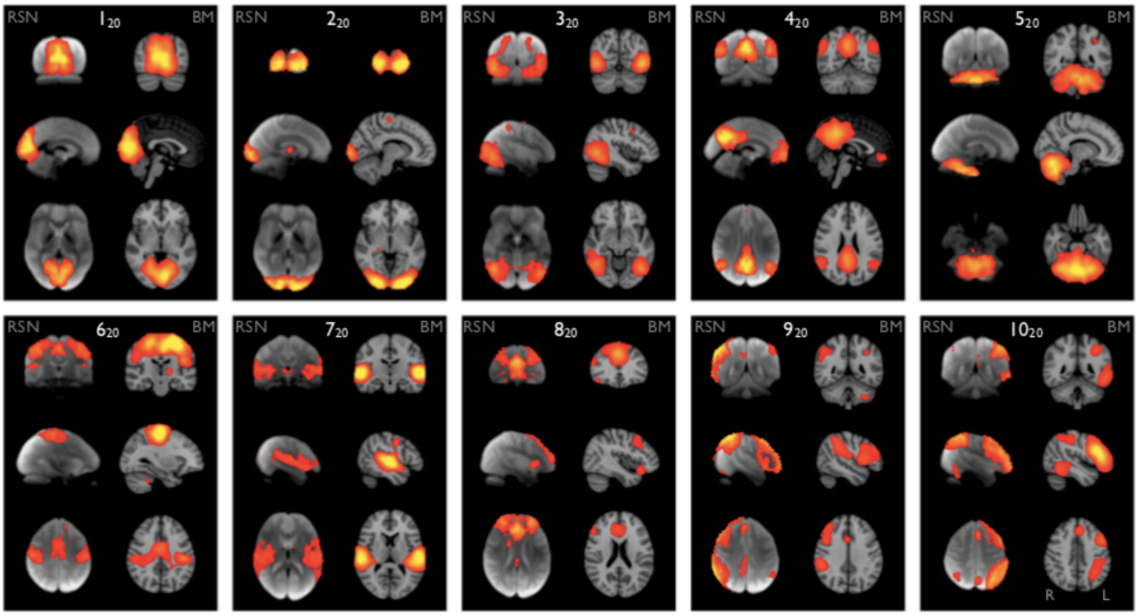


Figure 1 The ten resting state networks established by Smith et al.. In 2009, Smith and colleagues identified the major activation networks by carrying out an analysis of thousands of separate activation maps derived from the BrainMap database of functional imaging studies, involving nearly 30,000 human subjects. Left column: resting fMRI data, superimposed on the mean images from the 36-subject resting FMRI dataset. Right column: corresponding network from the 29,671-subject BrainMap activation database, superimposed on the MNI152 standard space template image. Reprinted from *'Correspondence of the brain's functional architecture during activation and rest'* by Smith et al., 2009, Oxford: Oxford University Press, Copyright by Oxford University Press. 2009

Default Mode Network (DMN). Perhaps the most known resting state network is the Default Mode Network. Posterior cingulate cortex, precuneus, medial prefrontal cortex, inferior parietal lobule, and lateral temporal cortex comprise this network (Figure 2) (Bijsterbosch, Smith, & Beckmann, 2017). In the initial research years of the DMN, its peculiar activation was allotted to mental assignments in which the individual is not focused on the outside world, i.e., the brain at conscious rest, such as daydreaming and mind wandering (Raichle et al., 2001). It was hypothesized that internal cognitive processes needed to be down regulated in order to perform externally oriented behaviors, thus, negatively correlating the DMN with attention networks (Broyd et. al., 2009, Bijsterbosch, Smith, & Beckmann, 2017). Although it was originally reported that the DMN was inactive in goal-oriented tasks (coining the name of Task-Negative Network) (Fox et al., 2005), it has been evidenced in recent studies that it is active in social working memory and autobiographical tasks (Spreng, 2012). In 2018, Sormaz et al. discovered that this network also contributes to elements of external task experience. It is active when the subject is thinking of others (theory of mind, moral and social reasoning), thinking about oneself, remembering the past, episodic memory, planning for the future and story comprehension (Andrews-Hanna, 2011). In Andrews-Hanna's own words, the DMN plays an important role in the introspective and adaptive mental activities in which humans spontaneously and deliberately engage in everyday, i.e., internal mentation (ibid).

Figure 2 The three most representative orthogonal slices from the Default Mode Network, shown on a volumetric view. The network was identified using independent component analysis performed on data from the Human Connectome Project. Reprinted from *'Introduction to Resting State fMRI Functional Connectivity*', by Bijsterbosch, J., Smith, S., & Beckmann, C., 2017, Oxford: Oxford University Press, p.5. Copyright by Oxford University Press.

Sleep deprivation causes changes in connectivity within the default mode network (DMN). A sleep deprivation study found a significant reduced functional connectivity within the DMN, as well as a reduced anticorrelation between DMN and anti-correlated network (De Havas, 2012). The subjects of this study underwent full 24 hours of non-sleep. It's not further reported how do they measured the tiredness of the subjects, and if they took extra measures to prevent them from falling asleep during the scanning. This study supports the principle that sleep deprivation impacts the intrinsic connectivity within DMN and reduces anti-correlation between the DMN and anti-correlated network.

1.4. Hypothesis

The preceding paragraphs provide an overview of the most important theories that shaped this thesis, the overall effect observed in the resting brain after sleep deprivation, and how this affects pivotal brain networks. Functional changes have a direct impact on memory, social and emotional regions. The representative cases cited above provide supportive tools to investigate functional connectivity on sleep deprivation. This thesis has two purposes. First, we want to distinguish the well-established resting state networks in our group of healthy mature subjects. Second, we want to investigate possible associations between functional connectivity and lack of sleep in older people. To date, there is no available study that addresses functional connectivity in conjunction with partial sleep deprivation in a group of exclusively older people. Therefore, we also want to broaden the discoveries about younger subjects and apply that knowledge to a group between 65 and 75 years of age (inclusively). Based on the literature reviewed in this introductory chapter, we expect a decreased connectivity within the default mode network (De Havas et al., 2012).

2. Methods

2.1. Dataset

The dataset analyzed for this thesis was provided by the Stress Research Institute from the Stockholm University, via OpenfMRI (https://www.openfmri.org/dataset/ds000201/). Data included functional and structural MRI recordings. The study had a crossover within-group design. MRI scanning was performed twice with an interval of one month between sessions. The order of partial sleep deprivation or full sleep was randomized and counterbalanced. Participants slept in their own homes, monitored by ambulatory polysomnography. Full sleep condition group was instructed to follow their usual bedtime routine, while the sleep deprived group was instructed to go to bed three hours before the time they would usually wake up, and to continue their day's activities as usual. (Nilsonne, 2017).

2.2. Participants

The original sample by Nilsonne et. al. (2016) consisted on 36 mature (65-75 years) healthy participants. One participant was discarded because his number of volumes was smaller than the number of volumes of the other participants. 3 subjects reported to have fallen asleep during the resting state scan, thus, being discarded as well. 8 subjects presented a high-volume displacement, therefore being eliminated from the sample (Nilsonne 2017, Sörös 2019, Tamm 2019). The remaining data we worked with for the subsequent analysis consisted of 24 subjects. Mean age of the sample (n=24) was 71.6 years (range 65 – 75 years, SD= 3.2 years). 14 of the subjects were female.

2.3. Psychological Assessment

Sleepiness was measured with the Karolinska Sleepiness Scale (KSS) (Nilsonne et al., 2016). As reported in Nilsonne's manuscript, in order to select those participants who would be included in the sample, they were required to have no current or past self-reported psychiatric or neurological illness, including addictions, to not suffer from hypertension or diabetes, to not use psychoactive or immune-modulating drugs, to not smoke every day, and to not have a higher habitual daily caffeine intake greater than four cups of coffee. Further inclusion criteria included: no ferromagnetic items in body, no refractive error greater than five diopters, not to be color blind, right-handed, to be 65-75 years old (inclusive), understand and speak fluent Swedish, and to reside in the greater Stockholm area (Nilsonne et al., 2016).

The insomnia severity index (ISI) and the Karolinska Sleep Questionnaire (KSQ) were used to exclude participants with insomnia symptoms, out-of-circadian sleep patterns, or snoring/apnea (Nilsonne et al., 2016). The Insomnia Severity Index reports scores ranging from 0 to 28; a clinically relevant insomnia case is assessed by a result greater than or equal to 15 points (Morin, Belleville, Bélanger, & Ivers, 2011). The Karolinska Sleep Questionnaire (KSQ) was used to characterize sleep patterns and exclude participants who had a sleep phase that was too early, or too late, i.e., going to bed before 22:00pm, or after 1:00am. Further KSQ assessment criteria was not provided in the data. The Hospital Anxiety and Depression Scale (HADS) was used to exclude participants with depressive symptoms (Nilsonne et al., 2016). The Hospital Anxiety and Depression Scale reports scores ranging from 0 to 21; a result greater than or equal to 8 in either of the two mood disorders is considered a clinical case of anxiety and/or depression (Zigmond & Snaith, 1983). Further exclusion criteria included: students or employees in the fields of psychology, behavioral science, or medicine, nursing and allied fields. This background might be prone to the participants becoming aware of the experimental paradigm (Nilsonne et al., 2016). The participants were recruited through newspaper advertisements. To avoid behavior compensatory changes, such as naps, participants were not told until the evening before the experiment to what order they were randomized. Demographic information is shown in the table below.

Table 1

Demographic information from the included participants in this study.

Demographic Information	
Age (mean, range)	71.6 (65 - 75)
Sex (n male, n female)	10 , 14
BMI at first scanning (mean, SD)	24 (2.5)
Completed secondary education (n)	7
Currently enrolled in tertiary education (n)	1
Completed tertiary education (n)	16
ISI (mean, SD)	7 (1.5)
HADS (mean, SD)	1.5 (1.7)

Note. BMI= Body Mass Index. ISI= Insomnia Severity Index. HADS= Hospital Anxiety and Depression Scale.

2.4. Study Design

All the functional and anatomical images were acquired by the Sleepy Brain Project, who assessed functional connectivity in the resting state during two eight-minute runs in each session. The participants were instructed to stay awake while looking at a fixation cross presented against a gray background, displayed on special goggles. In the second run, participants rated their sleepiness with the Karolinska Sleep Scores (KSS) every two minutes. The second run was discarded from this study as rating their sleepiness may introduce task related noise. Participants were monitored regarding eye-tracking during fMRI scanning to ensure they were awake. The study has a cross-over within-group design. Participants slept at home while being monitored with ambulatory polysomnography. To capture the partial sleep deprivation condition, they were instructed to go to bed three hours before the time they wake up. For the full sleep condition, they were instructed not to change anything on their usual bedtime routine (Nilsonne et al., 2016).

2.5. Data Acquisition

Scanning was performed on a 3T Discovery 750 MRI scanner (General Electric) with an 8- channel head coil.

T1-weighted images were acquired with the following settings: TR 2.5s (2500ms), field of view 24, slice thickness 1 mm, sagittal orientation, interleaved acquisition bottom to top, covering the whole head. Before publication of images the face region was removed in order to preserve anonymity. (Nilsonne, 2017).

Resting state fMRI were obtained in accordance with the following procedure: echo-planar imaging (EPI) with field of view 28.8, slice thickness 3 mm, no interslice gap, axial orientation, 49 slices covering the whole brain, interleaved acquisition bottom -> up, TE 30ms, TR 2.5 s (2500ms), and flip angle 75° (Nilsonne, 2017).

2.6. Data Analysis

2.6.1. Preprocessing

The brain extraction was performed with the widely used Advanced Normalization Tools software (ANTs) (Avants, Tustison, & Song, 2011) on the anatomical images of both sessions, then the images with better quality were manually selected for further analysis. Preprocessing of resting state FMRI data was carried out using FMRIB's Software Library (FSL, version 6.00) (Smith et al., 2004, Jenkinson, Beckmann, Behrens, Woolrich & Smith, 2012). The following pre-statistics processing was applied: volume removal, head motion correction was performed by realignment to the middle using MCFLIRT

(Jenkinson et al., 2002). Because each subject had a different number of BOLD volumes, these were manually inspected, and the elimination of the first volumes was such that the outcome was 188 volumes, thus, allowing the balance of the signal. Non-brain removal using BET (Smith 2002); spatial smoothing using a Gaussian kernel of FWHM 5mm; grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor were applied. Based on previous literature (Nilsonne 2017, Sörös 2019, Tamm 2019), after MCFLIRT motion correction, each subject was individually analyzed, and those who presented a framewise displacement > 1.0 mm, equivalent to an amount greater than 25% of the total volumes, were discarded from this analysis, leading to the removal of 8 subjects.

Registration of functional to high resolution structural images was carried out using boundary-based registration in FLIRT (Jenkinson & Smith, 2001, Jenkinson, Bannister, Brady, & Smith, 2002). Registration from high resolution structural to Montreal Neurological Institute (MNI152) standard space was further refined using 12-parameter affine transformation and non-linear registration with a warp resolution of 12 mm in FNIRT (Anderson 2007a, 2007b). The denoised data sets were then high-pass filtered with a cutoff of 100s (0.01Hz).

2.6.2. ICA-Based Noise Removal

In resting state fMRI analysis, independent component analysis (ICA) is a commonly used method employed to decompose the 4-dimentional BOLD dataset into a space-structured set of components (Bijsterbosch, Smith & Beckmann, 2017). These components usually consist of signals, as well as noise. Because of this, in a data driven analysis, the first instance to use ICA will be to identify and remove artifactual noise. The second instance is when ICA is used in a group-level analysis to identify resting state networks.

In this paragraph the first occurrence will be detailed, while the second one will be further discussed in the dual regression section. ICA for noise reduction should be applied after standard preprocessing (motion correction, temporal filtering, and spatial smoothing) (Smith et al., 2004). Independent component analysis-based automatic removal of motion artifacts (FSL's ICA-AROMA version 0.3-beta) was employed to detect artifacts, and subsequently remove those components from the data. ICA yields a set of components that are composed of spatial maps and timecourses. The output is identified either as noise or as a neural signal. The program automatically separates the components into noise or signal. However, it is strongly recommended to do a visual inspection as well. There are 3 pieces of information: the spatial maps, the timecourses and the frequency spectra. Each of these tells a portion of the information about how to designate the component correctly. First, the spatial maps should exclusively overlap with the gray matter; if they overlap with white matter, cerebrospinal fluid, or show the characteristic motion “ring” around the brain, those components should be classified as noise. Second, the timeseries of a signal are stable, that is, no sudden changes in the oscillation pattern. And finally, the power spectrum should be in the low-frequency range (below 0.1 Hz) (Bijsterbosch, Smith & Beckmann, 2017, p.41). In order to remove the variance that corresponds to noise components, a regression analysis is performed (Beckmann, 2012). There are two options to proceed with this step: “aggressive” and “non- aggressive” removal. The aggressive approach eliminates all the variance explained by the noise component's timeseries, even if part of it is shared with signal components. The non-aggressive approach only removes the variance that solely belongs to the noise components, keeping the variance that is shared between noise and signal components (Bijsterbosch, Smith & Beckmann, 2017). The "non-aggressive" approach was used for this study in order to maintain the signal as much as possible (Sörös, 2019). Through multivariate exploratory linear decomposition into independent components, ICA-AROMA applies probabilistic ICA of each individual's resting state data (FSL’s MELODIC, version 3.15) (Beckmann & Smith, 2004). This tool employs temporal and spatial features to select motion-related components from the MELODIC output, removing them automatically from the initial data set (Pruim et al., 2015). ICA-AROMA is a robust and accurate approach to eliminate artifacts related to movement, in addition to preserving the signal of interest and increasing the reproducibility of resting state networks (Pruim, Mennes, Buitelaar, & Beckmann, 2015).

2.6.3. ICA as a voxel-based functional connectivity analyses

ICA is a data-driven (model free) exploratory data analysis method, which aims to decompose a multivariate signal into a set of features that represent structure in the given data, i.e., components. In other words, the objective of ICA is to separate the BOLD signal into different components, because it deduces that the given 4-D data is a mixture of multiple latent components that cannot be directly observed, but that can be separated (Beckmann, 2012, Bijsterbosch, Smith & Beckmann, 2017). It should also be noted that ICA is a multivariate approach, due to considering the data from all the voxels at once in order to find the components (ibid).

The components obtained with ICA are maps formed by spatial (the delineated space where the signal is located) and temporal (the timeseries that describes the pattern of the signal over time) properties (Beckmann & Smith, 2004, Beckmann & Smith, 2004b, Woolrich et al., 2009, Beckmann, 2012). Because its linearity, adding all the resulting components builds up the original BOLD data. ICA is widely used to extract resting state networks, such as the default mode network and dorsal attentional network. When ICA is applied to a resting state study, it has been demonstrated that these resting state networks can be found accurately and reliably (Smith et. al., 2009, Bijsterbosch, Smith & Beckmann, 2017, Beckmann, DeLuca, Devlin, & Smith, 2010).

2.7. Identifying the Components

Starting from the premise that the BOLD data is a set of mixed components, which can be divided due to their spatial and temporal characteristics, we proceed with the assertion that it is sufficient to separate the BOLD signals to obtain the components (Storti et al., 2013, Beckmann, DeLuca, Devlin, & Smith, 2010). In order to unmix the observed BOLD signal, it is necessary to factorize the data matrix (Bijsterbosch, Smith, & Beckmann, 2017). MELODIC is the tool in FSL that we use to separates the signal by looking for components that are maximally independent from each another (Smith et al., 2004), meaning that the program will look for components that are not correlated (there is no statistical association between them). When signals are statistically independent, signal ´A´ cannot be predicted due to signal 'B'. The best known example to clarify this concept is the throwing of two dice: the outcome of the first die cannot foretell the outcome of the second die. This is how this method is conducted to separate the resting state data into independent components (Bijsterbosch, Smith, & Beckmann, 2017). The margin of independence was applied on the spatial dimension. The spatial dimension (looking into spatially independent signals) was chosen over the temporal dimension (looking into temporally independent signals) due to two main reasons: 1) there are commonly more voxels than time points, 2) spatial signals are much more non- Gaussian (Beckmann, DeLuca, Devlin, & Smith, 2010., Beckmann, 2012). When the different signals are mixed, the average that forms such mixture has a more Gaussian distribution than the distribution of its signals separately. In other words, finding the set of components that are maximally non-Gaussian, allows to recognize the set of timecourses and independent spatial maps that explain the data (Bijsterbosch, Smith, & Beckmann, 2017).

2.8. Resting State Networks

To recognize significant networks caused by lack of sleep in older adults, all data sets (n = 24, preprocessed and de-noised as described above) were concatenated in temporal order to create a single data set. This concatenated data set was then decomposed into 30 spatially independent components using group ICA with MELODIC (In the next section, these 30 components will also be used as template maps for dual regression). As explained in previous paragraphs, ICA can be applied to identify and remove noise from a single subject data, as well as in a group level to identify large-scale resting state networks. Group ICA uses the denoised and preprocessed 4-D single subject data as an input. To extract the group components, first, all subject´s data are spatially registered, and thereafter temporally concatenated (all subjects together) (Beckmann et al., 2005). As shown in figure 3, the data of subject n+1 will be placed after the data of subject n, and so on, until a two-dimensional matrix (columns= timeseries, rows=space information) from all voxels is created.

30 components were deliberately selected because it has been demonstrated that in a data driven analysis conducted with the FSL tools (ICA MELODIC), a selection of less than 20 components can lead to a misestimation of the underlying signals (underfitting). On the other hand, it is known that estimating too many components (>50) leads to over-fitting, where some signals turned out to be divided and represented through multiple maps (Beckmann & Smith, 2004b, Beckmann, 2012, Woolrich et al., 2009). The literature is divided in an optimal fitting of 20 and 30 components, hence this analysis explored both for a better insight and comprehension of the possible results. It was identified that the 20-component run, predominantly presented superimposed maps, leaving relevant networks in disuse. The 30-component run yielded networks that were distinct, identifiable and comparable with the literature of Smith et al. (2009). These arguments are the basis on which a decomposition into 30 spatially independent components was properly chosen.

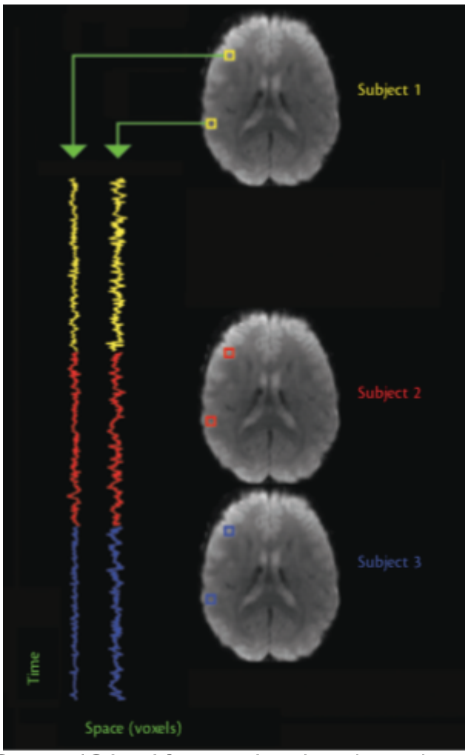


Figure 3 Concatenation Group ICA. After registering into the standard space, group-ICA is accomplished by merging all the data from the subjects. The data is temporally concatenated across subjects. Reprinted from *'Introduction to Resting State fMRI Functional Connectivity'*, by Bijsterbosch, J., Smith, S., & Beckmann, C., 2017, Oxford: Oxford University Press, p.59. Copyright by Oxford University Press.

To explore possible resemblances between the components from this study and the well-established components from Smith, a spatial cross-correlation between them was performed using FSL's fslcc command. Five canonical resting state networks were selected from the resulting data, that showed a spatial affinity with the well-established RSNs published by Smith et al. in 2009, thus, improving the extent of analysis and visualization of this study (Figure 5 shown in results).

2.9. Dual Regression

The interest of this study is not only to identify the resting states networks, but also to carry out a statistical analysis that allows us to compare between groups, answering the question about a feasible change related to lack of sleep. To address this comparison, FSL's dual regression script was used. Dual regression is a powerful script that extracts the timeseries for each ICA map in a resting state analysis (Filippini et al., 2009, Nickerson, Smith, Öngür, & Beckmann, 2017). It is named 'dual' because it involves two stages of multiple regression (GLM), aiming to capture spatial-maps components (Bijsterbosch, Smith, & Beckmann, 2017). The data input into both stages is the preprocessed BOLD data from subject n (done one subject at a time). The model input in stage 1 is the set of components derived from the group ICA. The output of stage 1 is the subject´s n timeseries for each group component, subsequently used as input model for stage 2. The outcome of the second stage is a spatial map that will be used for a group-level analysis (Figure 4) (Nickerson, Smith, Öngür, & Beckmann, 2017). (Comparing differences in network structure between subjects is what Bijsterbosch et al. (2017) call stage 3 of dual regression).

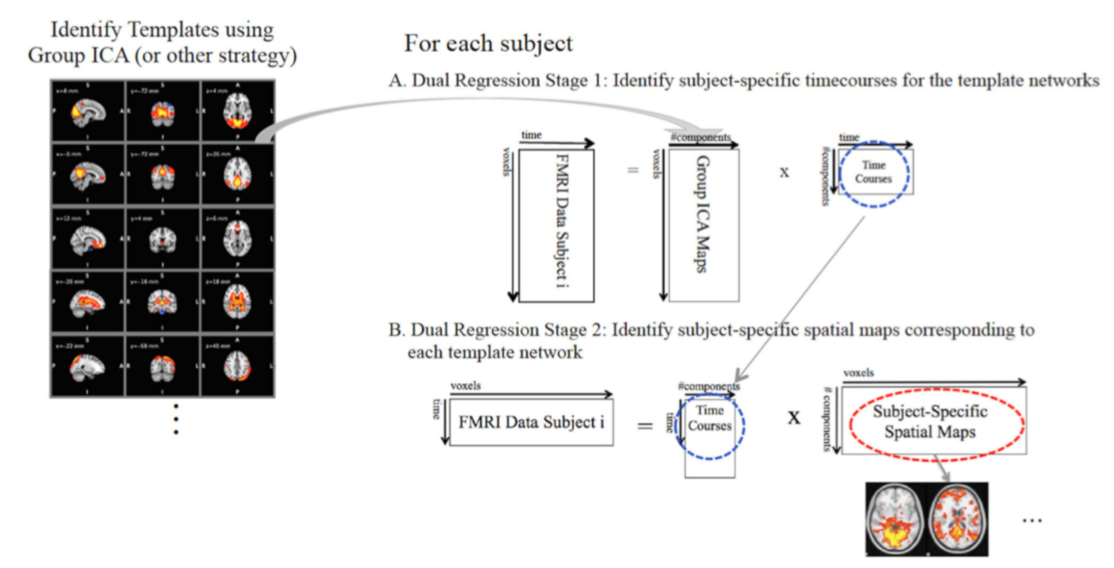


Figure 4 Dual Regression Stages. A) FIrst stage, the template maps are regressed against each subjects FMRI data in order to extract subject-specific timecourses. B) Second stage, the output of the stage 1 (subject-specific timecourses) is regressed against the subjects FMRI data, resulting in a set of subject-specific spatial maps. Adapted from `*Using Dual Regression to Investigate Network Shape and Amplitude in Functional Connectivity Analyses'* by Nickerson, Smith, Öngür, & Beckmann, 2017. Copyright by Nickerson et al. 2017

The 30 ICA components were regressed in the first stage of dual regression against each participant's 4 dimensional rs-fMRI data, producing 30 time series per participant (one for each template map). In the second stage, the subject-specific time series from stage 1 are regressed against the subjects 4 dimensional rs-fMRI data to identify each participant's specific spatial maps (corresponding to the 30 map templates) (Nickerson, Smith, Öngür, & Beckmann, 2017).

To identify possible effects related to the sleep condition within the 30 networks, a paired two-sample t-test was performed on the participant-specific spatial maps for each network using the general linear model (Sörös, 2019). FSL's randomise (version 2.9) was used with 10,000 permutations for a non-parametric permutation testing (Nichols & Holmes, 2001). Family wise error rate was applied (FWE) with $p < 0.05$.

Table 2. *Methodological processes used in FSL (FMRIB's Software Library)*

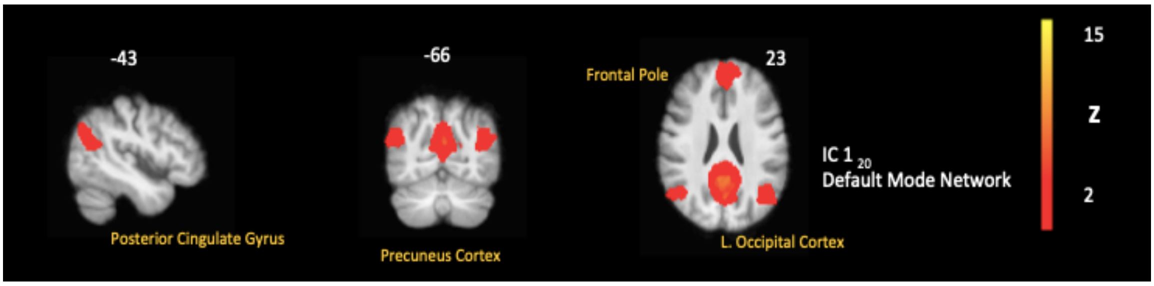
Steps:	Tool:	Analysis Methods:	Reference:
Preprocessing of anatomical images; Brain extraction	**ANTS**	Brain extraction and bias correction (makes images more homogeneous)	(Avants, Tustison, & Song, 2011)
General preprocessing of functional images	**FEAT (FMRI Expert Analysis Tool)**	Non-brain removal; spatial smoothing using a Gaussian kernel of FWHM 5mm; grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor.	(Smith, 2002)
Removal of volumes and ascertainment of Framewise displacement <1.0mm	**MCFLIRT (contained in FEAT)**	Volume removal, head motion correction done through realignment to the middle. Elimination of the first volumes was such that the product was 188 volumes. After MCFLIRT motion correction, each subject was individually analyzed, and those who presented a framewise displacement > 1.0 mm, equivalent to an amount greater than 25% of the total volumes, were discarded from this analysis, leading to the removal of 8 subjects	(Jenkinson et al., 2002) (Nilsonne 2017, Sörös 2019, Tamm 2019)
Registration	**FLIRT** **FNRT** **(both contained in FEAT)**	FLIRT: Linear registration of functional to high resolution structural images. FNRT: Non linear registration. Registration from high resolution structural to Montreal Neurological Institute (MNI152) standard space was further refined using 12-parameter affine transformation and non-linear registration with a warp resolution of 10 mm	(Jenkinson & Smith, 2001, Jenkinson, Bannister, Brady, & Smith, 2002). (Anderson 2007a, 2007b).
Artifact removal / motion artifacts	**ICA AROMA** **MELODIC ICA**	Used to identify and remove motion-related ICA components from fMRI data. Employs temporal and spatial features to select motion-related components, and removes these from the initial data set.	(Beckmann & Smith, 2004) (Pruim et al., 2015)
Identification of networks	**GROUP MELODIC ICA**	To identify networks, MELODIC uses the previous ICA-AROMA component outputs, concatenates them in temporal order and creates a single data se, which is later decomposed into the 30 spatially independent components.	(Beckmann & Smith, 2004)
Seeks for associations between the networks	**DUAL REGRESSION**	1.- The 30 components identified by group ICA were regressed against each participant's 4-dimensional rs-fMRI data set, producing 30 time series per participant (one for each template map). 2.- The subject-specific time series from stage 1 are regressed against the subjects 4 dimensional rs-fMRI data to identify each participant's specific spatial maps (corresponding to the 30 map templates).	(Filippini et al., 2009, Nickerson, Smith, Öngür, & Beckmann, 2017).

3. Results

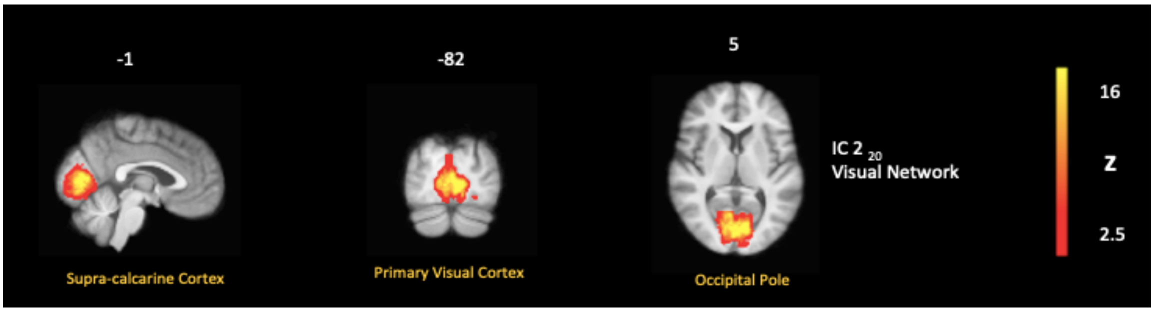
3.1. Resting State Networks

Once the decomposition was performed using MELODIC, 5 resting state networks were found using group level analysis within the general linear model (GLM) framework, coinciding with previous published studies (Smith et. al., 2009). Figure 5 illustrates the 5 resting state networks identified in our sample of older adults (components 1, 2, 6, 8, and 17) from the 30-component prototype as a result of the group ICA. Figure 5 shows the 3 most informative orthogonal slices from the network. All ICA spatial maps were converted to z statistic images (ibid), thresholded at Z 2. The resting state networks were confined in the MNI space using mask analysis as defined by Smith et al. (2009), thus, allowing to identify the networks and its regions. The correlated spontaneous fluctuations identified when the brain is at rest (not performing a task) have a correspondence to the major functional networks (from the task-engaged brain) (Smith et al., 2009).

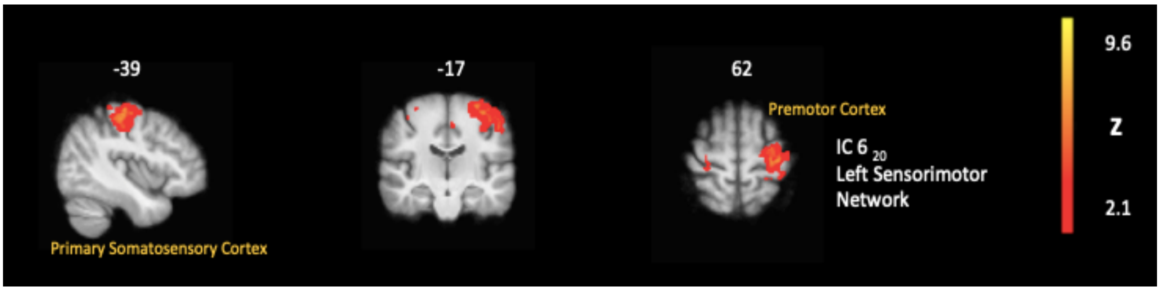
a) IC1 Default Mode Network



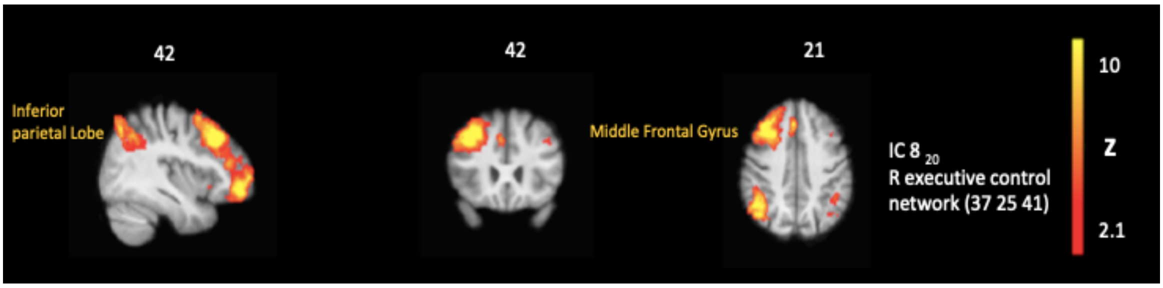
b) IC2 Visual Network



c) IC6 Left Sensorimotor Network



d) IC8 Right Executive Control Network



e) IC17 Cerebellar Network

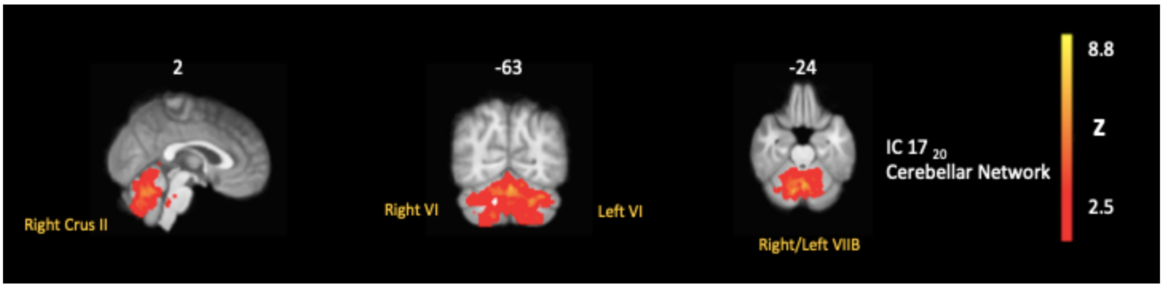


Figure 5 (a - e) The 5 resting state networks identified in our sample of older adults from the 30 component prototype as a result of the group ICA, coinciding with the well-established networks identified by Smith et. al., 2009. Brain images are displayed in radiological convention (right hemisphere appears on the left side, positive sagittal axes= right side). IC = Independent Component.

Map 1_{30} Default Mode Network (medial and parietal precuneus; posterior cingulate; frontal cortex (Smith et. al. 2009)). Probably the most studied network in the resting state FMRI literature. One of the first DMN assertions was that it is most active when we are not engaged in a task (Raichle et al., 2001). Further literature suggests that it sustains internal cognitive processes, representations of the self (Buckner et al., 2008), some degrees of consciousness (Laureys et al., 2004), and it is even linked to self-awareness, past memories, and projections to the future (Addis et al., 2007).

Map 2_{30} Visual Network (medial and occipital pole; lateral visual areas; primary visual cortex (Smith et. al. 2009, Nikolaou et al., 2016)). This map is consistent to the visual behavioral domain. Cognition–language–orthography correspond to the occipital pole whereas cognition–space correspond to the lateral visual maps (Smith et al. 2009). In this study, collecting the resting state scans was prior to any task. Nevertheless, the slightest visual exposure can trigger an activity on this network (Rosazza & Minati, 2011). A probable explanation can be the visual novelty of being inside a magnetic resonance machine, and the use of the googles.

Map 6_{30} Sensorimotor Network (somatosensory and motor regions, and supplementary motor areas (Biswal et al., 1995, Smith et. al. 2009)). The sensorimotor network was the first identified resting state network, when back in 1995, Biswal et al. found activations in bimanual motor tasks (Biswal et al., 1995, Smith et. al. 2009).

Map 8_{30} Right Executive Control Network (several frontal areas, regions of the posterior/inferior parietal lobules (Smith et. al. 2009)). This network pertains to well-known cognitive paradigms, such as perception–somesthesis–pain, emotion and action–inhibition (ibid). In addition, it has been documented that the Executive Control Network is involved in tasks that require external attention, such as working memory, task-switching, and data integration (Beaty et al., 2015).

Map 17_{30} Cerebellar Network (Covers the cerebellum (Smith et. al., 2009)). This network is considered to be involved in action-execution and perception–somesthesis–pain circuits (ibid).

Differences in functional connectivity between genders. An 2 x 2 ANOVA showed no significant interaction between male and female, not under full sleep condition, neither under partial sleep deprivation condition.

Associations between functional connectivity and sleepiness. A two-sample paired t-test with the Karolinska Sleepiness Scores as a regressor of interest produced two contrasts: normal sleep > sleep deprived and sleep deprived > normal sleep. Neither revealed significant results in functional connectivity in this sample.

3.2. Association Between Functional Connectivity and Sleep Deprivation

The components mentioned above were used as an input in the general linear model, an endeavor to answer the question whether a lack of sleep affects the identified networks. A two-sample paired t-test was performed, yielding significant results for the normal sleep > partially sleep deprived contrast, revealing regions that show a decreased cerebellar connectivity in the partially sleep deprived group compared to the normal sleep condition. Moreover, the second contrast, partially sleep deprived > normal sleep, did not reveal any significant differences, confirming that the relevant brain changes occur after partial sleep deprivation. In other words, reduced sleep decreases connectivity within the cerebellar network in a group of healthy mature subjects. Figure 6 shows the significant decrease in functional connectivity, found in the cerebellar network, IC 17. The cluster encompasses the Left VI ($k = 1273$, $p < 0.01$), the coordinates of the voxel with highest significance are: $x = -14$ mm, $y = -64$ mm, $z = -22$ mm. Table 3 contains the MNI peak coordinates and the scope of the local maxima.

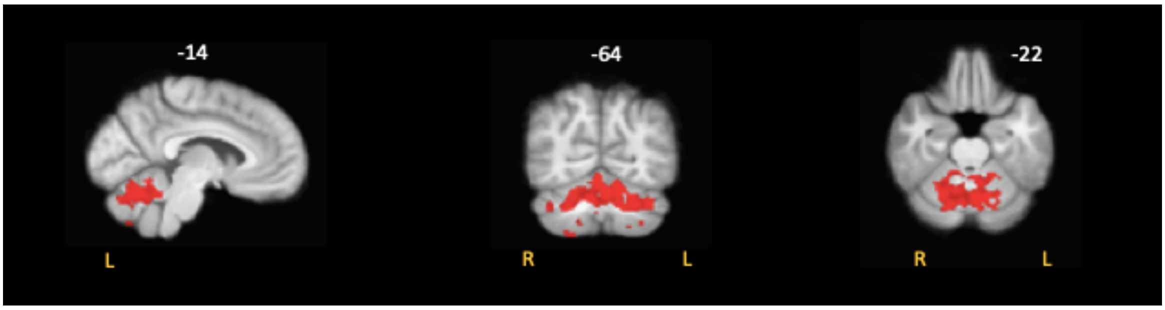


Figure 6 Region of decreased functional connectivity in a healthy group of sleep deprived mature subjects (65-75 years) within the cerebellar network (IC17 in figure 4). The MNI coordinates of the voxel with highest significance are: x=-14, y=-64, z=-22 (p= 0.01). Brain images are displayed in radiological convention.

Table 3

Region of decreased functional connectivity in an elderly population after one night of partial sleep deprivation in the cerebellar network.

Cluster Index	Region	No. of voxels	x(mm)	y(mm)	z(mm)	p-value
6	**Left VI (95%)**	**1273**	**-14**	**-64**	**-22**	**0.01**

Cerebellar nomenclature is based on Schmahmann et al. (2000), in his work he labeled and divided the cerebellar lobules from I-X, from the anterior/superior border, to the anterior/inferior border. FSL tools include a probabilistic atlas of the human cerebellum, based on Schmahmann et al. (2000), which was produced by averaging the cerebellar lobule masks of 20 subjects, aligned to the standard MNI152 space (creating two different atlases, one with affine registration and one with non-linear registration) (Diedrichsen et al., 2009, 2011).

4. Discussion

4.1. Key Findings and Interpretations

The aim of this study was to better understand partial sleep deprivation in an elderly group, using resting state fMRI analysis. To this end, this thesis arrived at two major findings. First, it demonstrates the feasibility of producing canonical networks in a group level decomposition on resting state data. It obtained five networks, which show spatial affinity with the claims of Smith (2009). Second, based on the pertinent literature, this study expected to find a decreased connectivity within the default mode network (De Havas et al., 2012). On the contrary, it did not obtain any significant changes in the mentioned network. In contrast, it is worth noticing that this thesis found a decreased activity in the cerebellar network (Left VI), as originating from the partially sleep deprived condition.

Pursuant to the definition of functional connectivity, the internal communicative capacity of the cerebellar network is diminished after a partially sleep deprived night. In other words, Intrinsic functional networks are evidenced by the slow spontaneous fluctuations that are correlated when the brain activity is at ‘rest’. Thus, the results suggest that the degree of regional cerebellar covariation is decreased due to a lack of sleep.

4.2. The Relevance of the Cerebellum in the Results

The cerebellum is not directly involved with sleep, but rather through its cerebro-cortical pathways (Manto, Gruol, Schmahmann, Koibuchi, & Rossi, 2012). According to the current understanding, the functionality of the cerebellar network resides in relation to the joint work it performs with cerebral brain regions. Despite the fact that the cerebellar network is accepted as one of the established networks, there still exist no conclusive insight into the intra-connectivity properties that this network is supposed to have.

Due to the unfeasibility of anatomical analyses, cerebellar connections remained mostly unmapped until the study of cerebellar connectivity took a new direction when researchers started using resting state analysis methods (O'Reilly, Beckmann, Tomassini, Ramnani, & Johansen-Berg, 2009). Based on a resting state analysis, O´Reilley and colleagues divided the human cerebellum into two main zones: the primary sensorimotor zone (Lobules V, VI, and VIII) and the supramodal zone (Crus I and II). The former is functionally connected to the motor and premotor cortex, somatosensory cortex, and some visual and auditory regions, while the latter is functionally connected to the prefrontal and parietal cortex. Figure 7 shows the cerebellar correlation from the motor and prefrontal cortices as presented by O'Reilley and colleagues. The authors also concluded that the cerebro-cerebellar mapping is contralateral, meaning that the right-hemisphere of the cerebral cortex had a stronger correlation in the left cerebellum, and vice versa (O'Reilly et al., 2009). These results are coherent with Schmahmann's assertions, where the cerebellum is divided into motor and executive regions (Schmahmann and Sherman 1998). Nevertheless, there is not an exclusive association between the executive functions described by the authors and one distinct region of the cerebral cortex. Thus, cerebellar connectivity fits in a model where the cerebellum exhibits diverse functionality from (and to) a range of cortical regions, rather than being specialized in just one (O'Reilly et al., 2009).

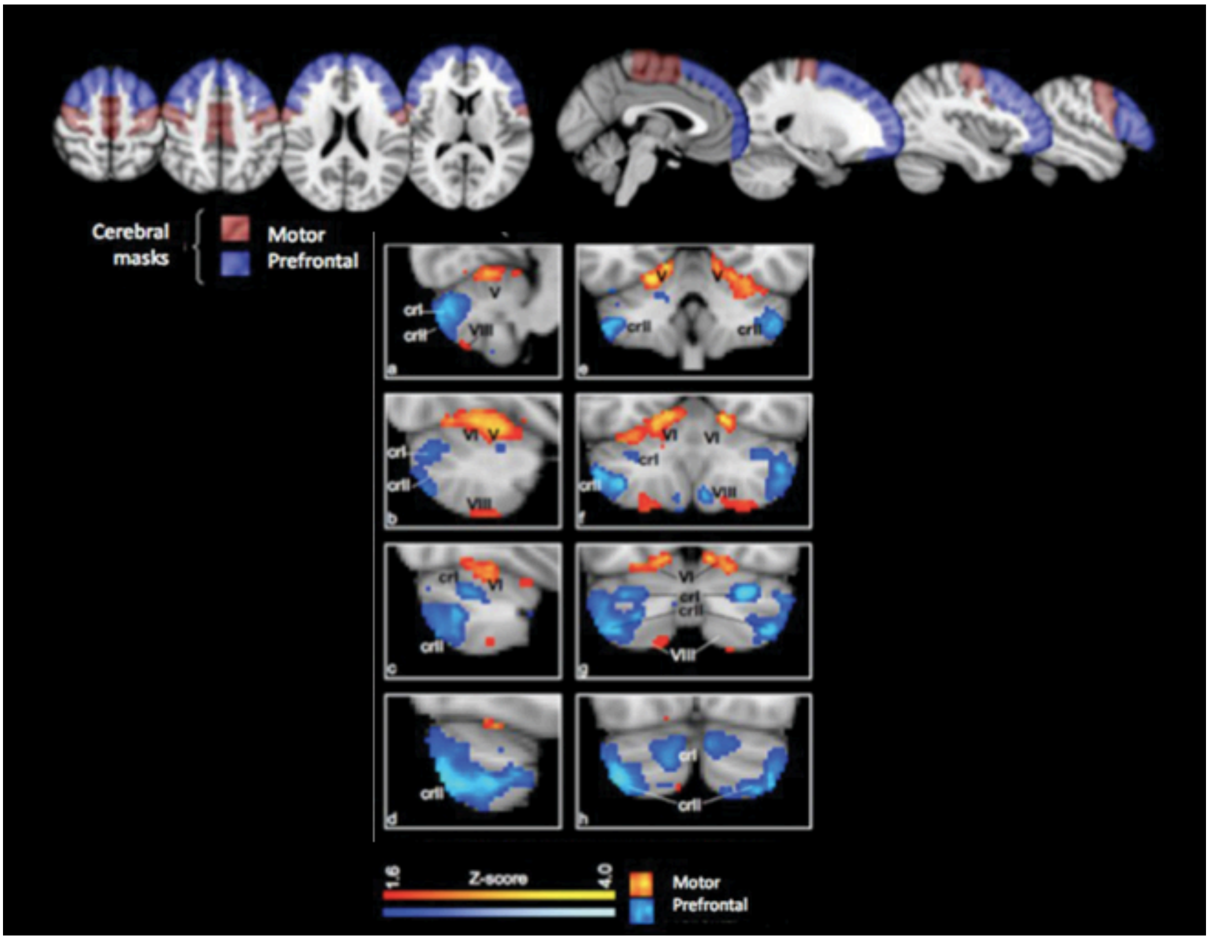


Figure 7 Correlation maps from motor and prefrontal cortices. Adapted from '*Distinct and Overlapping Functional Zones in the Cerebellum Defined by Resting State Functional Connectivity*', by O'Reilly, Beckmann, Tomassini, Ramnani, & Johansen-Berg, 2009. *Cerebral Cortex* 20 (4) p.956. Copyright 2009 by O'Reilly et al.

4.3. Cerebellum in Sleep Research

It is difficult to state with certainty the impact sleep deprivation has on the cerebellum, since to date, little is known about the interaction between the cerebellum during sleep (Canto, Onuki, Bruinsma, Van der Werf, & De Zeeuw, 2017). Cunchillos and De Andrés (1982), explained in their study, how cerebellar dysfunctions lead to important changes in the sleep-wakefulness cycle; DelRosso and Hoque (2014) also discovered in a recent study that a malfunction in the cerebellum impairs sleep; additionally, Rath, Rohde, and Møller (2012) revealed that clock genes (circadian rhythm associated) are expressed in cerebellar neurons as well. Last but not least, primary malfunctions of the cerebellum are generally accompanied by sleep disorders and vice versa (Canto et al., 2017).

Research has shown a decrease in cerebellar activity during the transitional phase from awake (pre- sleeping) to slow wave sleep (SWS) (Braun, as cited in Canto, 2017). This is an observation of direct relevance to the results of this study, since the findings of this thesis coincide with the characteristics of Braun's claim. Another study suggests that the mental phase prior to falling asleep is similar to the state a sleep-deprived subject experiences (Vetrugno & Montagna, 2011). Until today, no specific moment has been identified as defining the sleep onset. It is rather characterized by the sublime and gradual changes in behavioral and physiological characteristics, such as EEG rhythms, cognition, mental processing, and reaction time (Chokroverty, 2017). The sleep onset begins even before stage 1 NREM, epitomized by heavy eyelids, blurry senses, as well as a distorted perception of the external stimuli (ibid). This phase was coined by McDonald Critchley as 'pre-dormitum'. According to Vetrugno & Montagna, these are the same somnolence indicators as caused by more than one cycle being awake. Interestingly, there exist specialized studies on this pre-dormitum phase, since some motor abnormalities, such as restless leg syndrome, usually occur at this stage (Vetrugno & Montagna, 2011). In fact, this could be coherent with the decreased activity in the cerebellar anterior lobe given by this thesis. Indeed, much literature can be found linking this sleep stage with motor discrepancies. The mentioned study is not in realm of functional connectivity, and this thesis lacks physiological data. These comparable resemblances cannot be more than a possible interpretation. Future studies attempting to solve these compelling congruencies will need both physiological data as well as rs-fMRI.

Patients with an acute case of REM sleep behavior disorder have a decreased cerebellar volume (DelRosso & Hoque, 2014). In general, diverse studies suggest that patients who suffer from sleep disorders typically present a lower cerebellar volume (Canto et al., 2017, Cunchillos & De Andrés, 1982, DelRosso & Hoque, 2014). It is well known that normal and healthy aging comes with a decrease in the volume of the brain and cerebellum. Given the age range of the subjects included in this study, it is very likely that their cerebellum was already smaller in volume than the one they had in their youth. However, to verify this, a volumetric analysis would be necessary. Therefore, when studying the connectivity schema in older adults, future studies could take the volumetric measurement into account.

4.4. Conclusion & Outlook

The subsequent deliberation considers, to what degree one can infer a verifiable impact on sleep deprivation by solely a decreased connectivity in the posterior lobe of the cerebellum. As mentioned above, the existing literature on cerebellar functional connectivity connotes that the extent of this network's capabilities depends on the connections it has with the brain. In other words, the scope of the cerebellar network is delimited by its concerted functions with the brain. The cerebellum is an organ of versatile functionality, and this feature is better appreciated when examining its extensive connectivity with the motor and cognitive brain regions (Ren, Guo & Guo, 2019). The observation obtained by the mere decrease in cerebellar spontaneous fluctuations is not enough to confidently address the impact of sleep deprivation on this healthy group of mature adults. To be able to objectively report the manifolds implications that this cerebellar network raises, it would also be necessary to examine the areas of the brain that work in conjunction with the cerebellum -such as the frontal cortex and thalamus, which are involved in the fronto-cerebellar network, and the motor and prefrontal cortices, as shown in O´Reilley, 2009- , and thus be able to verify whether there is a functional impact caused by the lack of sleep, or not. This not only applies to the cerebellum, but also to the different regions that, to date, are known to suffer a decrease in functional connectivity as a result of lack of sleep. The results of this study are demarcated by the chosen connectivity analysis. It should be remembered that, due to the essence of connectivity using a data-driven method, very specific hypotheses could not have been supported. In addition, the existing literature has not yet addressed partial sleep deprivation in a group of mature people using resting state functional connectivity analysis. Thus, finding deactivations in the cerebellar network is a profit.

Another important point concerns the difficult interpretation of a crossover within-group design of only healthy subjects. This healthy group was partially sleep deprived for only one night. Of course, sleeping three hours gives an ecological validity to this study that allows to simulate a realistic restlessness night. Nilsonne also argued in favor of the three-hour sleep measure in order to decrease the tendency of the subjects to fall asleep in the scanner. Nonetheless, three hours could be sufficient to achieve a restful sleep in a healthful group, biasing the primary concern of what a sleep deprivation analysis would require (Tamm, 2019).

This study has the potential to open a path to the preliminary question of how sleep deprivation influences functional brain connectivity. Using other analytical tools that can delve into more specific regions could broaden not only the interpretation of results, but also the factors presented in the initial research question. By utilizing ICA/dual regression, we are bound to detect changes only within the resting state networks proposed by Smith in 2009. Seed-based could be a complementary analysis to expand to other brain regions, such as the thalamus and amygdala, which are known to be affected by lack of sleep. Previous studies have already employed seed-based analysis in the field of sleep deprivation. Shao and colleagues deprived their subjects of sleep for a period of 36 hours. Employing seed-based analysis, their study revealed distributed changes in the thalamocortical connectivity (Shao et al., 2013, 2014). In another study, Lei and colleagues demonstrated that sleep deprivation causes changes in connectivity from the amygdala, specially decreased connectivity between amygdala and prefrontal cortex (Lei et al., 2015). Future studies could be based on these analyses and apply them to a group of mature people as well.

The performed analysis in this thesis was merely exploratory and fulfilled its investigative function satisfactorily. It addressed the fact that the ICA methodology approaches the underlying BOLD signals, prime factor in the resting state maps. Its voxel-wise applicability to the whole brain makes ICA a powerful tool for resting state fMRI, and the temporal signals from the resting state maps can be easily set apart for group comparison. Some of the advantages of ICA-based noise removal are the following: it is the perfect tool to work with when researching resting state fMRI because it is a data-driven method; detects noise components from a vast range of sources, including physiological noise and MRI artifacts (Smith et al., 2004, Bijsterbosch, Smith & Beckmann, 2017). In contrast, some ICA-based noise removal disadvantages are its ability to separate components is highly dependent on the temporal and spatial resolution of the data. The more time points, the better. It is more difficult to detect noise components in a relatively low-quality data; it also depends on the division of noise and signal components (Bijsterbosch, Smith & Beckmann, 2017).

What is evident is that the study of resting state networks has been, and will continue to be, of high value for scientific dissemination. It provides a significant insight on how spontaneous connectivity patterns are altered under different settings. As a further factor of consideration, the precise connotation of these intrinsic processes, fundamental to the neural functional architecture, is still uncertain. One of the main premises of the resting state study is that, due to the removal of task driven BOLD changes, it would contribute to the optimal baseline of brain function. Yet, even Smith and Beckmann, in their 2009 study, mention that its unconstrained constitution yields so varied interpretations that it can fall into an outlook difficult to interpret.

This study substantiates the previous findings on resting state functionality. Further endeavors are needed to support the main factors of our initial hypothesis. Future research could use a seed-based analysis in conjunction with ICA/ dual regression to directly target regions known for being affected by lack of sleep, and to search for changes within the well-established resting state networks. Additionally, a potential aim might be a comparison of a healthy control group (such as the group we work with), and a group that presents difficulties in their sleep patterns; behavioral data may be a very useful addition to this research, allowing to elucidate better on the impact of partial sleep deprivation. Now that we have some evidence of irregularities in the cerebellum, this study could lead to an analysis between a control group and a group that presents motor abnormalities related to poor sleep.

5. References

Addis, D. R., Wong, A. T., & Schacter, D. L. (2007). Remembering the past and imagining the future: Common and distinct neural substrates during event construction and elaboration. *Neuropsychologia*, *45*(7), 1363–1377. https://doi.org/10.1016/j.neuropsychologia.2006.10.016

Analysis Group, FMRIB, Oxford, UK.. (2010). Correspondence between BrainMap and Resting-FMRI ICA components. Retrieved August 5, 2019, from https://www.fmrib.ox.ac.uk/datasets/brainmap+rsns/

Andersson, J.L.R., Jenkinson, M., & Smith S.M. (2007) Non-linear optimisation. *FMRIB technical report* TR07JA1.

Avants, B. B., Tustison, N., & Song, G. (2011). *Advanced Normalization Tools (ANTS)*. Retrieved from http://scil.dinf.usherbrooke.ca/static/website/courses/imn530/ants.pdf

Beckmann, C. F., DeLuca, M., Devlin, J., & Smith, S. M. (n.d.). *Investigations into Resting-state Connectivity using Independent Component Analysis* (FMRIB Technical Report TR05CB1). Retrieved from https://www.fmrib.ox.ac.uk/datasets/techrep/tr05cb1/tr05cb1.pdf

Beckmann, C., & Smith, S. (2004). Probabilistic Independent Component Analysis for Functional Magnetic Resonance Imaging. *IEEE Transactions on Medical Imaging*, *23*(2), 137–152. https://doi.org/10.1109/tmi.2003.822821

Beckmann, C. F. (2012). Modelling with independent components. *NeuroImage*, *62*(2), 891–901. https://doi.org/10.1016/j.neuroimage.2012.02.020

Beckmann, C. F., DeLuca, M., Devlin, J. T., & Smith, S. M. (2005). Investigations into resting-state connectivity using independent component analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1457), 1001–1013. https://doi.org/10.1098/rstb.2005.1634

Berry, R. et al. (2015) The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology, and Technical Spec- ifications Version 2.2, American Academy of Sleep Medicine

Bijsterbosch, J., Smith, S., & Beckmann, C. (2017). Introduction to Resting State fMRI Functional Connectivity. Oxford, United Kingdom: Oxford University Press.

Biswal, B., Zerrin Yetkin, F., Haughton, V. M., & Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar mri. *Magnetic Resonance in Medicine*, *34*(4), 537–541. https://doi.org/10.1002/mrm.1910340409

Broyd, S. J., Demanuele, C., Debener, S., Helps, S. K., James, C. J., & Sonuga-Barke, E. J. (2009). Default-mode brain dysfunction in mental disorders: A systematic review. *Neuroscience & Biobehavioral Reviews*, *33*(3), 279–296. https://doi.org/10.1016/j.neubiorev.2008.09.002

Buckner, R. L., Andrews-Hanna, J. R., & Schacter, D. L. (2008). The Brain's Default Network. *Annals of the New York Academy of Sciences*, *1124*(1), 1–38. https://doi.org/10.1196/annals.1440.011

Castellanos, F. X., Margulies, D. S., Kelly, C., Uddin, L. Q., Ghaffari, M., Kirsch, A., . . . Milham, M. P. (2008). Cingulate-Precuneus Interactions: A New Locus of Dysfunction in Adult Attention-Deficit/Hyperactivity Disorder. *Biological Psychiatry*, *63*(3), 332–337. https://doi.org/10.1016/j.biopsych.2007.06.025

Chokroverty, S. (2017). Sleep Disorders Medicine: Basic Science, Technical Considerations and Clinical Aspects. New York, United States of America: Springer New York.

Cunchillos, J., & De Andrés, I. (1982). Participation of the cerebellum in the regulation of the sleep-wakefulness cycle. Results in cerebellectomized cats. *Electroencephalography and Clinical Neurophysiology*, *53*(5), 549–558. https://doi.org/10.1016/0013-4694(82)90067-0

D'Mello, A. M., & Stoodley, C. J. (2015). Cerebro-cerebellar circuits in autism spectrum disorder. *Frontiers in Neuroscience*, *9*. https://doi.org/10.3389/fnins.2015.00408

DelRosso, L. M., & Hoque, R. (2014). The Cerebellum and Sleep. *Neurologic Clinics*, *32*(4), 893–900. https://doi.org/10.1016/j.ncl.2014.07.003

De Havas, J. A., Parimal, S., Soon, C. S., & Chee, M. W. (2012). Sleep deprivation reduces default mode network connectivity and anti-correlation during rest and task performance. *NeuroImage*, *59*(2), 1745–1751. https://doi.org/10.1016/j.neuroimage.2011.08.026

Diedrichsen, J., Balsters, J. H., Flavell, J., Cussans, E., & Ramnani, N. (2009). A probabilistic MR atlas of the human cerebellum. *NeuroImage*, *46*(1), 39–46. https://doi.org/10.1016/j.neuroimage.2009.01.045

Diedrichsen, J., Maderwald, S., Küper, M., Thürling, M., Rabe, K., Gizewski, E., . . . Timmann, D. (2011). Imaging the deep cerebellar nuclei: A probabilistic atlas and normalization procedure. *NeuroImage*, *54*(3), 1786–1794. https://doi.org/10.1016/j.neuroimage.2010.10.035

Duffy, J. F., Willson, H. J., Wang, W., & Czeisler, C. A. (2009). Healthy Older Adults Better Tolerate Sleep Deprivation Than Young Adults. *Journal of the American Geriatrics Society*, *57*(7), 1245–1251. https://doi.org/10.1111/j.1532-5415.2009.02303.x

Filippini, N., MacIntosh, B. J., Hough, M. G., Goodwin, G. M., Frisoni, G. B., Smith, S. M., . . . Mackay, C. E. (2009). Distinct patterns of brain activity in young carriers of the APOE-ε4 allele. *Proceedings of the National Academy of Sciences*, *106*(17), 7209–7214. https://doi.org/10.1073/pnas.0811879106.

Fox, M. D., & Raichle, M. E. (2007). Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nature Reviews Neuroscience*, *8*(9), 700–711. https://doi.org/10.1038/nrn2201

Fox, M. D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Van Essen, D. C., & Raichle, M. E. (2005). From The Cover: The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proceedings of the National Academy of Sciences*, *102*(27), 9673–9678. https://doi.org/10.1073/pnas.0504136102

Goel, N., Rao, H., Durmer, J., & Dinges, D. (2009). Neurocognitive Consequences of Sleep Deprivation. *Seminars in Neurology*, *29*(04), 320–339. https://doi.org/10.1055/s-0029-1237117

Harrison B.J., Pantelis C. (2010) Cognitive Subtraction. In: Stolerman I.P. (eds) Encyclopedia of Psychopharmacology. Springer, Berlin, Heidelberg

Hong, C. C., Harris, J. C., Pearlson, G. D., Kim, J., Calhoun, V. D., Fallon, J. H., . . . Pekar, J. J. (2009). fMRI evidence for multisensory recruitment associated with rapid eye movements during sleep. *Human Brain Mapping*, *30*(5), 1705–1722. https://doi.org/10.1002/hbm.20635

Jenkinson, M., Bannister, P., Brady, M., & Smith, S. (2002). Improved Optimization for the Robust and Accurate Linear Registration and Motion Correction of Brain Images. *NeuroImage*, *17*(2), 825–841. https://doi.org/10.1006/nimg.2002.113

Jenkinson, M., Beckmann, C. F., Behrens, T. E., Woolrich, M. W., & Smith, S. M. (2012). FSL. *NeuroImage*, *62*(2), 782–790. https://doi.org/10.1016/j.neuroimage.2011.09.015

Jenkinson, M., & Smith, S. (2001). A Global Optimisation Method for Robust Affine Registration of Brain Images. *Medical Image Analysis*, *5*(2), 143–156. https://doi.org/10.1016/s1361-8415(01)00036-6

James, W. (1870). *The Principles of Psychology*. New York, USA: H. Holt and Company, 1890.

Kelly, R. M., & Strick, P. L. (2003). Cerebellar Loops with Motor Cortex and Prefrontal Cortex of a Nonhuman Primate. *The Journal of Neuroscience*, *23*(23), 8432–8444. https://doi.org/10.1523/jneurosci.23-23-08432.2003

Konrad, K., & Eickhoff, S. B. (2010). Is the ADHD brain wired differently? A review on structural and functional connectivity in attention deficit hyperactivity disorder. *Human Brain Mapping*, *31*(6), 904–916. https://doi.org/10.1002/hbm.21058___

Laureys, S., Owen, A. M., & Schiff, N. D. (2004). Brain function in coma, vegetative state, and related disorders. *The Lancet Neurology*, *3*(9), 537–546. https://doi.org/10.1016/s1474-4422(04)00852-x

Lei, Y., Shao, Y., Wang, L., Ye, E., Jin, X., Zou, F., . . . Yang, Z. (2015). Altered superficial amygdala-cortical functional link in resting state after 36 hours of total sleep deprivation. *Journal of Neuroscience Research*, *93*(12), 1795–1803. https://doi.org/10.1002/jnr.23601

Manto, M., Gruol, D. L., Schmahmann, J., Koibuchi, N., & Rossi, F. (2012). *Handbook of the Cerebellum and Cerebellar Disorders*. Amsterdam, Netherlands: Springer Netherlands.

Morin, C. M., Belleville, G., Bélanger, L., & Ivers, H. (2011). The Insomnia Severity Index: Psychometric Indicators to Detect Insomnia Cases and Evaluate Treatment Response. *Sleep*, *34*(5), 601–608. https://doi.org/10.1093/sleep/34.5.601

Nichols, T. E., & Holmes, A. P. (2001). Nonparametric permutation tests for functional neuroimaging: A primer with examples. *Human Brain Mapping*, *15*(1), 1–25. https://doi.org/10.1002/hbm.1058

Nickerson, L. D., Smith, S. M., Öngür, D., & Beckmann, C. F. (2017). Using Dual Regression to Investigate Network Shape and Amplitude in Functional Connectivity Analyses. *Frontiers in Neuroscience*, *11*. https://doi.org/10.3389/fnins.2017.00115

Nikolaou, F., Orphanidou, C., Papakyriakou, P., Murphy, K., Wise, R. G., & Mitsis, G. D. (2016). Spontaneous physiological variability modulates dynamic functional connectivity in resting-state functional magnetic resonance imaging. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *374*(2067), 20150183. https://doi.org/10.1098/rsta.2015.0183

Nilsonne, G., Tamm, S., D'Onofrio, P., Thuné, H., Schwarz, J., Lavebratt, C., . . . Åkerstedt, T. (2016). A multimodal brain imaging dataset on sleep deprivation in young and old humans: The Stockholm Sleepy Brain Study. Recovered from http://hdl.handle.net/10616/45181

Nilsonne, G., Tamm, S., Schwarz, J., Almeida, R., Fischer, H., Kecklund, G., . . . Åkerstedt, T. (2017). Intrinsic brain connectivity after partial sleep deprivation in young and older adults: results from the Stockholm Sleepy Brain study. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-09744-7

O'Reilly, J. X., Beckmann, C. F., Tomassini, V., Ramnani, N., & Johansen-Berg, H. (2009). Distinct and Overlapping Functional Zones in the Cerebellum Defined by Resting State Functional Connectivity. *Cerebral Cortex*, *20*(4), 953–965. https://doi.org/10.1093/cercor/bhp157

Pruim, R. H., Mennes, M., Van Rooij, D., Llera, A., Buitelaar, J. K., & Beckmann, C. F. (2015). ICA-AROMA: A robust ICA-based strategy for removing motion artifacts from fMRI data. *NeuroImage*, *112*, 267–277. https://doi.org/10.1016/j.neuroimage.2015.02.064

Pruim, R. H., Mennes, M., Buitelaar, J. K., & Beckmann, C. F. (2015). Evaluation of ICA-AROMA and alternative strategies for motion artifact removal in resting state fMRI. *NeuroImage*, *112*, 278–287. https://doi.org/10.1016/j.neuroimage.2015.02.063

Oxford Dictionaries | English. (2019). *network | Definition of network in English by Oxford Dictionaries*. [online] Available at: https://en.oxforddictionaries.com/definition/network [Accessed 3 Jun. 2019].

Raichle, M. E. (2010). Two views of brain function. *Trends in Cognitive Sciences*, *14*(4), 180–190. https://doi.org/10.1016/j.tics.2010.01.008

Raichle, M. E., MacLeod, A. M., Snyder, A. Z., Powers, W. J., Gusnard, D. A., & Shulman, G. L. (2001). A default mode of brain function. *Proceedings of the National Academy of Sciences*, *98*(2), 676–682. https://doi.org/10.1073/pnas.98.2.676

Rath, M. F., Rohde, K., & Møller, M. (2012). Circadian Oscillations of Molecular Clock Components in the Cerebellar Cortex of the Rat. *Chronobiology International*, *29*(10), 1289–1299. https://doi.org/10.3109/07420528.2012.728660

Ren, Y., Guo, L., & Guo, C. C. (2019). A connectivity-based parcellation improved functional representation of the human cerebellum. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-45670-6

Rosazza, C., & Minati, L. (2011). Resting-state brain networks: literature review and clinical applications. *Neurological Sciences*, *32*(5), 773–785. https://doi.org/10.1007/s10072-011-0636-y

Schmahmann, J., & Sherman, J. C. (1998). The cerebellar cognitive affective syndrome. *Brain*, *121*(4), 561–579. https://doi.org/10.1093/brain/121.4.561

Shao, Y., Wang, L., Ye, E., Jin, X., Ni, W., Yang, Y., . . . Yang, Z. (2013). Decreased Thalamocortical Functional Connectivity after 36 Hours of Total Sleep Deprivation: Evidence from Resting State fMRI. *PLoS ONE*, *8*(10). https://doi.org/10.1371/journal.pone.0078830

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E., Johansen-Berg, H., . . . Matthews, P. M. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage*, *23*, 208–219. https://doi.org/10.1016/j.neuroimage.2004.07.051

Smith, S. M., Fox, P. T., Miller, K. L., Glahn, D. C., Fox, P. M., Mackay, C. E., … Beckmann, C. F. (2009). Correspondence of the brain's functional architecture during activation and rest. Proceedings of the National Academy of Sciences of the United States of America, 106(31), 13040–13045. doi:10.1073/pnas.0905267106

Sonuga-Barke, E. J., & Castellanos, F. X. (2007). Spontaneous attentional fluctuations in impaired states and pathological conditions: A neurobiological hypothesis. *Neuroscience & Biobehavioral Reviews*, *31*(7),977-986. https://doi.org/10.1016/j.neubiorev.2007.02.005.

Sormaz, M., Murphy, C., Wang, H., Hymers, M., Karapanagiotidis, T., Poerio, G., . . . Smallwood, J. (2018). Default mode network can support the level of detail in experience during active task states. *Proceedings of the National Academy of Sciences*, *115*(37), 9318–9323. https://doi.org/10.1073/pnas.1721259115

Spreng, R. N. (2012). The Fallacy of a “Task-Negative” Network. *Frontiers in Psychology*, *3*. https://doi.org/10.3389/fpsyg.2012.00145

Sörös, P., Hoxhaj, E., Borel, P., Sadohara, C., Feige, B., Matthies, S., . . . Philipsen, A. (2019). Hyperactivity/restlessness is associated with increased functional connectivity in adults with ADHD: a dimensional analysis of resting state fMRI. *BMC Psychiatry*, *19*(1). https://doi.org/10.1186/s12888-019-2031-9

Sripada, C., Kessler, D., Fang, Y., Welsh, R. C., Prem Kumar, K., & Angstadt, M. (2014). Disrupted network architecture of the resting brain in attention-deficit/hyperactivity disorder. *Human Brain Mapping*, *35*(9), 4693–4705. https://doi.org/10.1002/hbm.22504

Storti, S. F., Formaggio, E., Nordio, R., Manganotti, P., Fiaschi, A., Bertoldo, A., & Toffolo, G. M. (2013). Automatic selection of resting-state networks with functional magnetic resonance imaging. *Frontiers in Neuroscience*, *7*. https://doi.org/10.3389/fnins.2013.00072

Sudre, G., Szekely, E., Sharp, W., Kasparek, S., & Shaw, P. (2017). Multimodal mapping of the brain’s functional connectivity and the adult outcome of attention deficit hyperactivity disorder. *Proceedings of the National Academy of Sciences*, *114*(44), 11787–11792. https://doi.org/10.1073/pnas.1705229114

Tamm, S. (2019). A Neuroimaging Perspective On The Emotional Sleepy Brain. Stockholm, Sweden: Karolinska Institutet.

Tomasi, D., & Volkow, N. D. (2011). Gender differences in brain functional connectivity density. *Human Brain Mapping*, *33*(4), 849–860. https://doi.org/10.1002/hbm.21252

Vetrugno, R., & Montagna, P. (2011). Sleep-to-wake transition movement disorders. *Sleep Medicine*, *12*, 11–16. https://doi.org/10.1016/j.sleep.2011.10.005

Woolrich, M. W., Jbabdi, S., Patenaude, B., Chappell, M., Makni, S., Behrens, T., . . . Smith, S. M. (2009). Bayesian analysis of neuroimaging data in FSL. *NeuroImage*, *45*(1), 173–186. https://doi.org/10.1016/j.neuroimage.2008.10.055

Zhou, X., Wu, T., Yu, J., & Lei, X. (2017). Sleep Deprivation Makes the Young Brain Resemble the Elderly Brain: A Large-Scale Brain Networks Study. *Brain Connectivity*, *7*(1), 58–68. https://doi.org/10.1089/brain.2016.0452

Zigmond, A. S., & Snaith, R. P. (1983). The Hospital Anxiety and Depression Scale. *Acta Psychiatrica Scandinavica*, *67*(6), 361–370. https://doi.org/10.1111/j.1600-0447.1983.tb09716.x

Declaration of Originality

I hereby confirm that this thesis is entirely my own work. I confirm that no part of the document has been copied from either a book or any source -including the internet- except where such sections are clearly shown as quotations and the sources have been correctly identified within the text or list of references. Moreover I confirm that I have taken notice of the 'Leitlinien guter wissenschaftlicher Praxis' of the University of Oldenburg.

Place, Date Signature