Brain Networks & Dynamics in Narcolepsy

Natasha Morales Drissi

Brain Networks and Dynamics in Narcolepsy

Natasha Morales Drissi

Linköping University medical dissertations, No. 1651
Department of Medical Health and Sciences (IMH)
Centre for Medical Image Science and Visualization (CMIV)
Linköping University, Sweden
Linköping 2018

Cover page: "Scattering mind"

Date of defense: 2019-01-25

ISBN: 978-91-7685-181-4

ISSN: 0345-0082

Printed by LiU-Tryck, Linköping 2018

To Gabriel and Noah

"Seeing the world through your eyes is a delightful joy,

I love you all the way to the restaurant at the end of the universe and back"

Populärvetenskaplig sammanfattning

Narkolepsi är en kronisk sömnsjukdom där den drabbade lider av obetvinglig sömnighet. Det gör att personer med sjukdomen blir mycket trötta under dagen och får sömnattacker som inte går att förhindra. Till symtomen hör också kataplexi som innebär plötslig kraftlöshet och en känsla av förlamning i samband med känslor som glädje och ilska. En del får hallucinationer strax innan de somnar eller vaknar.

Narkolepsi delas in i två typer. Typ 1 beror på en autoimmun reaktion som innebär att kroppens immunsystem angriper de hjärnceller som producerar ett hormon som reglerar vakenhet och sömn. Personer som fick symptom efter att ha vaccinerats med vaccinet Pandemrix har narkolepsi typ 1. Narkolepsipatienter beskriver också ofta andra icke-sömnrelaterade besvär, så som svårigheter med koncentration och minnet, men också kraftig viktuppgång trots att de inte äter mer. I den här avhandlingen har en grupp narkolepsipatienter 13-20 år med narkolepsi typ 1 undersökts med magnetkamera som har mätt hjärnans struktur och aktivitet, samt undersökt sammansättning av kroppsfett.

Brunt fett, också kallat aktivt fett, för att det ökar ämnesomsättningen påverkas i djur som har narkolepsi och vi trodde att det kunde vara brist på brunt fett hos narkolepsipatienter som gör att de går upp i vikt. Resultaten från mätningen av kroppsfett visade att narkolepsipatienterna inte hade mindre brunt fett än motsvarande friska individer. Däremot så hade de högre bukfetma, även om förhållandet mellan det "dåliga" fettet (som ökar risk för hjärt och kärlsjukdomar och diabetes typ 2) och det mer neutrala underhudsfettet var bättre hos narkolepsipatienterna.

Resultaten visar också att narkolepsipatienter har flera avvikelser i de områden i hjärnan som behandlar uppmärksamhet. Detta påverkar troligtvis deras förmåga att ta in information och kan upplevas som att de har "dålig minne". Ett av de områden som vi fann avvikelser i har möjligheter för att kunna användas som en ny behandling för koncentrationssvårigheter i narkolepsi typ 1.

Abstract

Narcolepsy is a chronic sleep disorder, characterised by excessive daytime sleepiness with frequent uncontrollable sleep attacks. In addition to sleep-related problems, changes in cognition have also been observed in patients with narcolepsy and have been linked to the loss of Orexin-A in a number of studies. Results from previous functional and structural neuroimaging studies would suggest that the loss of Orexin-A has numerous downstream effects in terms of both resting state glucose metabolism and perfusion and reduction in cortical grey matter. Specifically, studies investigating narcolepsy with positron emission tomography (PET) and single photon emission computed tomography (SPECT) have observed aberrant perfusion and glucose metabolism in the hypothalamus and thalamus, as well as in prefrontal cortex. A very recent PET study in a large cohort of adolescents with type 1 narcolepsy further observed that the hypo- and hypermetabolism in many of these cortico-frontal and subcortical brain regions also exhibited significant correlations with performance on a number of neurocognitive tests. These findings parallel those found in structural neuroimaging studies, where a reduction of cortical grey matter in frontotemporal areas has been observed.

The Aim of this thesis was to investigate mechanisms and aetiology behind the symptoms in narcolepsy through the application of different neuroimaging techniques. I present in this thesis evidence supporting that the complaints about subjective memory deficits in narcolepsy are related to a misallocation of resources.

I further describe how this has its seat in defective default mode network activation, possibly involving alterations to GABA and Glutamate signaling. In addition to this, I present our findings of a structural deviation in an area of the brainstem previously not described in the aetiology of narcolepsy.

This finding may have implications for further understanding the aetiology of the disease and the specific neuronal populations involved.

In addition to this, I show evidence from adipose tissue measurements in specific compartments, confirming that weight gain in narcolepsy is characterised by centrally located weight gain and may be specifically related to OX changes, but maybe not brown adipose tissue volume.

Acknowledgments

I would first like to thank my thesis advisor Professor Maria Engström. The door to your office was always open whenever I had a question about my research or writing. You have consistently allowed any paper to be my own work, yet steered me in the right the direction whenever you thought I needed it.

And to my Co-supervisors Associate Professor Gunnar Cedersund for providing me with the connections that allowed me to fulfil my long-time dream of living in Japan and Professor Fredrik Elinder for always being a good sport whenever I needed that signature at the last minute.

I would also like to acknowledge Dr. Suzanne T. Witt for all the support with my project and for always so generously (and patiently) sharing your knowledge. Most importantly, helping me (finally) understand what an ANOVA is and why there are so very many numbers in SPSS!

A special thanks to Dr. Helene Veenstra as the second reader of this thesis, I am gratefully indebted to you for your valuable comments.

I would also especially like to thank, nurses, and nurse aids at CMIV. All of you have been there to support me when I recruited patients and collected data for my Ph.D. thesis. I especially want to thank Christer for never hesitating to share with me your vast knowledge of EEG in the form of constructive criticism.

And everyone else at CMIV for the cheer and camaraderie during lunch and “fika,” especially Roz, Sebastian, Anette, Marcel, Thobias, Sofie and Marcus I will miss ALL of our discussions.

Dr. Karin Lundengård, for giving me the honour of being your Toastmaster, it is unfortunate you had to leave before you had a chance to reciprocate. Thank you for also letting me inherit your discarded office decorations. They are my precious.

I’d also like to thank everyone at Funahashi lab at Keio University, Hiyoshi Campus, for all the great memories. It gave me the energy I sorely needed to finish this thesis.

A special thanks to all my family. Words cannot express how grateful I am to my mother, for all of the sacrifices that you've made on my behalf. For always insisting that I can do anything and be anything, that voice at the back of my head has been the one to pick me up when I was ready to give up and also to my "bonus"-father for your unrelenting optimism.

To my late father Liborio Angelo Morales, I carry your name with pride.

To mormor and Philip. Not a day goes by when I don't feel your presence in my mind, I am fortunate to have had your love. You are sorely missed.

To my sister Alexandra for always refusing to listen to anything even remotely related to my research. You gave me the tenacity to continue talking even when no one cares to listen.

To my "bonus"-sisters Eva for injecting spontaneity into my life and Elin, for being such an inspiring role model.

I would also like to thank my friend Becky, who years ago set an image in my mind of working hard to achieve ones goal. It has been my inspiration throughout the writing of this thesis.

Finally, I must express my very profound gratitude to my husband for providing me with unfailing love, support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. For everything you do for your family everyday, I appreciate you and this accomplishment would not have been possible without you Anass. Thank you.

Supervisor:

Maria Engström

Professor

Department of Medical Health and Sciences

Centre for Medical Image Science and Visualization (CMIV)

Linköping University

Co-Supervisors:

Gunnar Cedersund

Associate Professor

Department of Biomedical Engineering

Department of Clinical and Experimental Medicine

Linköping University

Fredrik Elinder

Professor

Department of Clinical and Experimental Medicine

Linköping University

Opponent:

Birgitte Kornum

Associate Professor

Faculty of Health Sciences, University of Copenhagen

Copenhagen, Denmark

Summary of Papers

I. Altered Brain Microstate Dynamics in Adolescents with Narcolepsy

Natasha Morales Drissi, Attila Szakacs, Suzanne T. Witt, Anna Wretman, Martin Ulander, Henrietta Ståhlbrandt, Niklas Darin, Tove Hallböök, Anne-Marie Landtblom, Maria Engström

II. Evidence for Cognitive Resource Imbalance in Adolescents with Narcolepsy

Suzanne T. Witt, Natasha Morales Drissi, Sofie Tapper, Anna Wretman, Attila Szakacs, Tove Hallböök, Anne-Marie Landtblom, Thomas Karlsson, Peter Lundberg, Maria Engström

III. Structural Anomaly in the Reticular Formation in Narcolepsy Type 1, Suggesting Specific Damage to the Locus Coeruleus

Natasha Morales Drissi, Marcel Warntjes, Alexander Wessén, Attila Szakacs, Tove Hallböök, Niklas Darin, Anne-Marie Landtblom, Helena Gauffin, Maria Engström

IV. Unexpected Fat Distribution in Adolescents with Narcolepsy

Natasha Morales Drissi, Thobias Romu, Anne-Marie Landtblom, Attila Szakacs, Tove Hallböök, Niklas Darin, Magnus Borga, Olof Dahlqvist Leinhard, Maria Engström

Abbreviations

BOLD	Blood Oxygen Level Dependent
dHb	deoxy-Hemoglobin
DMN	Default Mode Network
EEG	Electroencephalography
fMRI	functional Magnetic Resonance Imaging
GABA	γ -Aminobutyric Acid
Hb	Hemoglobin
MRI	Magnetic Resonance Imaging
oHb	oxy-Hemoglobin
OX	Orexin
BAT	Brown Adipose Tissue
BFCS	Basal Forebrain Cholinergic System
qMRI	Quantitative Magnetic resonance Imaging
LC	Locus Coeruleus
CSF	Cerebrospinal Fluid
RSNs	Resting State Networks
H-MRS	Proton Magnetic Resonance Spectroscopy
ACC	Anterior Cingulate Cortex
LMFG	left-middle-frontal-gyrus
mPFC	Medial Pre-frontal Cortex

Table of Contents

1. Introduction	3
1.1 A Short History of Narcolepsy	3
Von Economo and Encephalitis Lethargica	4
1.2 A Short Introduction to Sleep	5
2. Narcolepsy	7
2.1 Clinical Features	7
2.2 Aetiology and Pathophysiology	7
Hypocretin/Orexin	8
Cataplexy and Excessive Daytime Sleepiness	8
Genetics and Immunology	9
Vaccine	10
2.3 Diagnostic Criteria	10
3. Obesity in Narcolepsy	13
3.1 Orexin and Obesity	13
3.2 Brown Adipose Tissue	13
4. Cognitive Differences in Narcolepsy	15
Default Mode Network in Attention	16
5. Methods	17
5.1 EEG	17
EEG microstates	17
5.2 Magnetic resonance imaging	19
Relaxation Rates	20
T1 and T2	20
Measuring Fat and Water	21
Different MRI Images	21
Functional Magnetic Resonance Imaging	21
Task-Based fMRI	22
Resting State fMRI	22

Simultaneous EEG and fMRI	24
Measuring GABA and Glutamate	24
6. Aims	27
7. Results	29
7.1 Brain Function in Narcolepsy	29
Microstates-Resting State fMRI	29
Working Memory and Sustained Attention	32
7.2 Brain Structure and Connectivity	35
Connectivity	37
7.3 Orexin and brown adipose tissue	38
8. Discussion	39
8.1 Cognitive Complaints in Narcolepsy	39
8.2 Obesity and Sleep	40
8.3 Potential Targets for Treatment and Biomarkers	41
Brainstem	41
Default Mode Network	42
Electroencephalography	43
8.4 Conclusion	43
References	45

“ ... a sleepy disposition—they eat and drink well, go abroad, take care well enough of their domestick affairs, yet whilst talking or walking, or eating, yea their mouthes being full of meat, they shall nod, and unless roused by others, fall fast asleep.”

Thomas Willis 1621-1675

1. Introduction

1.1 A Short History of Narcolepsy

The earliest account of narcolepsy comes from the scientific publications of Thomas Willis, a physician, and researcher in 17th century England. While he did not name the disorder, he proposed that it was humoral in origin and believed that the body was able to produce its own narcotic substance [1].

The word narcolepsy doesn't show up in the literature until two centuries later in the landmark papers published by French physician Jean-Baptiste-Edouard Gélinau in the journal '*La Gazette des Hôpitaux de Paris*'. His paper is the account of a wine merchant, afflicted throughout his adult life with somnolence and short sleep attacks. Gélinau recognised these symptoms as a clinical entity proposing the name 'narcolepsy', from the Greek words 'narke' (stupor, numbness) and 'lepsis' (attack, to seize), to describe this novel disease. He further postulated that it was a form of neurosis or functional condition, however, he did not make any special distinction about the functional origins of excessive sleepiness and other symptoms of the disease [2].

Prior to Gélinau, German physicians Westphal and Fisher had each published case studies describing excessive sleepiness together with sudden attacks of muscle weakness triggered by excitement in two patients, however, they did not believe in an organic nature of the disease and considered it to be a "disease of the mind". In fact, at the time, the leading hypothesis for pathological somnolence was heavily influenced by a famous case study of the serial murderer von Zastrov, who had suffered from pathological sleepiness. It was suggested that his symptoms were psychosomatic in nature, their expression believed to be a manifestation of repressed homosexuality and excessive masturbation [2].

The term cataplexy was first used by Löwenfeld in a paper from 1902, where he describes episodes of muscle weakness triggered by emotions and is taken from the Greek kataplexis (fixation of the eyes) [3]. Other symptoms such as sleep paralysis and hallucinations were later described by Kinnier Wilson [4], however it wasn't until the work by Yoss and Daly at the Mayo Clinic, that

these symptoms were all connected to the same disease and the canonical narcolepsy tetrad (EDS with sleep attacks, cataplexy, hallucinations, and paralysis) was first formed [5].

Von Economo and Encephalitis Lethargica

On April 17, 1917, at a meeting of the Vienna Society for Psychiatry and Neurology, Dr. Constantin von Economo presented a new disease he called 'encephalitis lethargica'. Cases had first emerged following the H1N1 "Spanish flu" epidemic that had raged through Europe and in 1917 von Economo published a series of patient cases on the disease describing it as a "sleeping sickness" with an unusually prolonged course. The epidemic encephalitis lethargica would last between 1917-1927 with an estimated mortality of 500 000 [6] and lead to a peaked interest in the study of sleep and its disorders including narcolepsy. At the time there still was no clear clinical definition of narcolepsy and in fact, the name was often used to refer to any type of pathologic somnolence. Encephalitis lethargica initially presented with a headache or malaise and just like in narcolepsy there were periods of somnolence and delirium. However, unlike with narcolepsy, it was not uncommon for the patient to die from the disease. For those that survived, followed apathy, lack of concentration, hypersexuality and compulsive behavior that led many to crime, symptoms which bring to mind the case of von Zastrov.

After studying patients suffering from encephalitis lethargica, many of which also developed narcolepsy with cataplexy, von Economo found that these patients had suffered an injury to the posterior hypothalamus. This led him to postulate that there must be a sleep center located in the posterior hypothalamus [7] and also speculated that the sleepiness seen in narcolepsy might have its origin in an injury to this area.

After the end of the epidemic, cases of encephalitis lethargica became rare and as cases declined so did the interest in studying them. The invention of electroencephalography (EEG) during this time was instrumental in defining the characteristics of the disease and it was first in 1999 that Lin *et al.* discovered that canine narcolepsy was caused by a mutation in a gene coding for a neurotransmitter peptide in the hypothalamus. This became the beginning of the new narcolepsy research.

1.2 A Short Introduction to Sleep

The idea that sleep is a biological phenomenon with specific neural pathways for regulating and initiating it did not start to take shape until the early 20th century. In 1924 the inventor of the EEG, German physiologist and psychiatrist Hans Berger recorded the first human EEG, its discovery enabled the quantitative study of brain activity, including sleep, and it became the basis for sleep stage classification [8].

Sleep occurs in repeating periods, which alternate between rapid eye movement sleep (REM) and non-REM (NREM) sleep over a period of 90 minutes, where a good night's sleep will typically contain 4–6 sleep cycles [9]. *The American Academy of Sleep Medicine* (AASM) divides sleep into four stages that are distinguished through their characteristics as measured by EEG. The whole sleep period normally proceeds in the order: N1 → N2 → N3 → N2 → REM (*Figure 1A*). N1 is the beginning of the sleep cycle and is a relatively light stage of sleep. It can be considered as a transitional period between sleep and wake, which typically lasts 5-10 minutes. A person that wakes up during N1 might not even report that they fell asleep at all. N1 is characterised by theta waves, which have a higher amplitude and lower frequency than the beta waves recorded during the wake stage. During N2, body temperature drops and breathing and heart rate become more regular. This lasts around 20 minutes and the EEG is characterised by short bursts of rapid rhythmic brain wave activity known as sleep spindles. On average, half of the normal night sleep is spent in N2 [9].

N3 is the stage in which the deepest sleep occurs, muscles relax and blood pressure drops. The EEG is characterised by high amplitude very low-frequency waves. REM sleep is characterised by rapid eye movement, increased respiration, and increased brain activity and about 20 percent of normal sleep is spent in this stage. During REM sleep, the brain becomes more active and it is also when most of the dreaming occurs, and voluntary muscles are immobilised to prevent movement during dreaming [9]. Sleep in narcolepsy is characterised by sleep onset REM periods (SOREMP), which are REM sleep periods that happen within 15 minutes of falling asleep [10], [11] and frequent awakenings throughout the night (*Figure 1B*).

Figure 1. Sleep Hypnogram. Shows approximate time spent in each sleep stage. A) Normal sleep progresses to REM sleep after first cycling through N1→N2→N3 and happens about 90 minutes after falling asleep. B) Narcolepsy with frequent awakenings and sleep onset REM period. W=Wake, REM=Rapid Eye Movement, N1=Sleep stage 1, N2= Sleep stage 2, N3= Sleep stage 3.

2. Narcolepsy

2.1 Clinical Features

Narcolepsy is characterized by excessive daytime sleepiness (EDS), which can lead to frequent "sleep attacks", causing patients to fall asleep uncontrollably during normal waking hours. Many patients also suffer from cataplexy, which is a sudden loss of skeletal muscle tone that is not accompanied by a loss of consciousness. Attacks of cataplexy are evoked by strong, often positive emotions, and can be debilitating. In addition to this narcolepsy patients also suffer from other sleep-related symptoms; such as hallucinations, which happen either at the time of falling asleep (hypnagogic) or at the time of waking up (hypnopompic), they can be very realistic and are sometimes also followed by sleep paralysis, making for potentially terrifying experiences ^[12].

It is also common for patients to suffer from sleep disturbances, like frequent awakenings and dissociated REM sleep ^[12] where the REM sleep will follow shortly after falling asleep instead of after a period of deep sleep. In fact, a so-called multiple sleep latency test (MSLT), where the time until entering REM sleep is measured is part of the diagnostic criteria ^[10]. Typical onset is from adolescence until early adulthood but there are also cases of narcolepsy in very young children ^[13], and some reports suggest that it may be even more common in preadolescence than previously considered. This is due to differences during early onset, making diagnosing younger children with narcolepsy more difficult as symptoms are often more severe in children and can also extend to problems not typically associated with narcolepsy such as attention deficits and aggressive behaviour ^{[10], [14]}.

2.2 Aetiology and Pathophysiology

Von Economo postulated, a century ago, that the observed symptoms in narcolepsy may have its seat in an injury to the hypothalamus. This has since been confirmed with the discovery of the loss of specialised cells in the hypothalamus of narcolepsy patients ^{[15]–[17]}.

Hypocretin/Orexin

In 1998, De Lecca et al. identified the pro-hormone pre-prohypocretin, and its peptide products, hypocretin (hcrt) -1 and -2, because of its structural similarity to secretin, as well as its localisation within the hypothalamus [18]. Within a month of this discovery, another group had published their finding of two novel peptides discovered using orphan receptor cloning, which they subsequently named orexin OX-A and -B (orexis from Greek appetite) after the appetite-stimulating effect it had in mice [19].

It soon became apparent that these were indeed the same peptides, however not until nomenclature had been established. There is still some contention as to which name should be used, often both will be mentioned.

Early research confirmed that OX is involved in the regulation of sleep [20], [21] ; and knocking out the OX gene in mice causes narcolepsy with cataplexy together with fragmented night sleep and sleep onset REM periods, similar to those displayed in humans with narcolepsy [22]. Additionally, the canine form of narcolepsy is caused by a mutation in the gene coding for the OX-A receptor [23]. Even though most human narcolepsy is not familial, low levels of OX-A have been confirmed in the cerebrospinal fluid (CSF) of patients suggesting similar disease mechanisms [24].

Further research revealed that OX knockout mice displayed no difference in the total amount of sleep as compared to their wild-type counterparts. Closer inspection of their sleep architecture did however reveal that it featured more transitions between different states of sleep and wakefulness. However, comparing other factors such as circadian control, sleep homeostatic control (i.e. if they can recuperate lost sleep) and arousal systems showed no difference between the knock out mice and the wild-type controls [25], suggesting that OX is important for behavioural state stability; specifically the waking state.

Cataplexy and Excessive Daytime Sleepiness

The use of different recent techniques to selectively activate OX neurones has enabled researchers to study the mechanisms behind this further. Designer receptors exclusively activated by designer drugs (DREADD) can be used to activate or suppress OX neuron signaling selectively. Using DREADD to excite orexin neurones specifically leads to a significant increase in the amount of time spent in wakefulness and decreases both NREM and REM sleep times in mice. Reverse inhibition of orexin neurones decreases wakefulness time and

increases NREM sleep time [26]. Optogenetics is another powerful tool in the study of neuron function, which allows for specific selective activation of neurones using photostimulation. Direct, selective, optogenetic photostimulation of OX neurones was shown to increase the probability of transition to wakefulness from either NREM or REM sleep [21].

Neuronal degeneration is not instantaneous but typically happens over a period of time, often following some sort of insult to the brain. Investigation of the different stages of neuronal degeneration reveals a relationship between a progressive loss of OX neurones and the reduced stability of long wake periods. Curiously while long wake bouts became less stable, the stability of short bouts of wake increased, in addition to increasing the chances of waking up during the first 30 seconds of NREM [27].

Together, these findings can help explain the sleepiness and fragmented sleep that are characteristic of narcolepsy. Further, restoration of signaling from populations of OX neurones within the hypothalamus revealed projection specific suppression of narcolepsy symptoms. In addition to this, suppression of cataplexy-like episodes correlated with the restoration of projections to the serotonergic neurones in the dorsal raphe [28], while the consolidation of fragmented wakefulness correlated with the restoration of projections to the noradrenergic neurones in the locus coeruleus [29].

One study found that the OX neurones activated during emotional and sensory-motor conditions also show burst discharge during phasic REM, suggesting that similar physiological mechanisms are activated during, at least part of, REM sleep and cataplexy [30].

Genetics and Immunology

While the exact cause behind the selective destruction of OX neurons in the hypothalamus of narcolepsy patients remains unknown, there is evidence suggesting the interplay of genetics and the immune system [31], [32]. Several immune-mediated mechanisms have been suggested in disease aetiology and an association between narcolepsy and the human leukocyte antigen (HLA) region, a region which codes for cell surface proteins important in regulating the immune system, was established in the late 1980s. Further studies by Mignot and colleagues also demonstrated that HLA DQA1*01:02 and HLA DQB1*0602 is highly associated with narcolepsy with cataplexy (narcolepsy type-1) within all ethnic populations [33] and is now considered a narcolepsy risk factor [34].

Most patients (76-98%) with narcolepsy type-1 and about half (40-60%) of patients with narcolepsy type-2 are positive for HLA DQB1*0602 [12], [35], however it is also carried by an estimated 20% of the normal population. Therefore, even though family aggregation exists in narcolepsy, HLA genes alone can't explain it and other non-HLA genes have also been shown to contribute to susceptibility to narcolepsy development [22].

Among those, strong associations have been found between narcolepsy and polymorphisms in the T-cell receptor α chain gene (TCR α), [31], [36] the protein that interacts with the HLA DQB1*0602 allele and plays an important role in recognition of antigen bound to HLA molecules [37]. These findings support the role of autoimmune-mediated processes in the pathogenesis of narcolepsy [38]. The role of the immune system in the pathogenesis of narcolepsy, lies outside the scope of this thesis, but has been comprehensively covered in recent reviews [34], [39], [40].

Vaccine

Following the 2009 H1N1 influenza pandemic, many new cases of narcolepsy were registered in Scandinavia and in 2010, a report from the Swedish Medical Product Agency suggested that narcolepsy could be a rare consequence of H1N1 influenza vaccination [41] and a subsequent similar finding was reported in Finland, indicating a possible link between a specific type of H1N1 vaccination (Pandemrix, ASO3-adjuvanted H1N1 vaccine) and early onset of narcolepsy [42]. Influenza has also been found to carry an increased risk of narcolepsy [43], interestingly; the timing of exposure to infection was critical such that the risk of developing narcolepsy increased in the ages prior to puberty. Evidence of the association between an elevation of streptococcal antibodies and the risk of developing narcolepsy has also been reported [43].

2.3 Diagnostic Criteria

The diagnostic criteria for narcolepsy in the American Academy of Sleep Medicine's *International Classification of Sleep Disorders* (ICSD) were recently updated in the ICSD-3 and narcolepsy is now divided into narcolepsy type 1 and narcolepsy type 2. A diagnosis of narcolepsy type-1 requires low levels of OX-A (under 110 pg/ml) and/or cataplexy, while narcolepsy type-2 patients have normal OX-A levels and no cataplexy [14].

Previously, narcolepsy was classified as with cataplexy or without cataplexy, however it has since been discovered that most narcolepsy patients with low OX-A will eventually develop cataplexy ^{[11], [44]}, even if this can take anywhere from a few weeks up to several decades after the first symptoms of EDS ^{[45], [46]}. Unlike narcolepsy type-1, the aetiology for narcolepsy type-2 is unknown, however research suggests that at least in a few cases the cause could be a less severe injury to the OX neurones ^[47], resulting predominantly in sleepiness and a small reduction in CSF hypocretin levels ^{[46], [48]}.

The presence of hallucinations (hypnopompic or hypnogogic), sleep paralysis and disturbed night sleep have also been removed from the diagnostic criteria as only 10% of narcolepsy patients will display the full tetrad of symptoms. Both narcolepsy type-1 and narcolepsy type-2 require the presence of EDS and a positive MSLT (≤ 8 minutes and 2 or more periods sleep onset REM periods).

These changes reflect an evolution away from a reliance on symptom recognition to a greater dependence on biomarkers and electrophysiological testing.

3. Obesity in Narcolepsy

3.1 Orexin and Obesity

Many patients with narcolepsy experience rapid weight gain ^[49], sometimes even in the face of hypophagia ^[50]. The evidence for this is not conclusive in humans, but is consistently observed in animal models ^[51]. It is a curious observation when we consider that OX-A was first named after its appetite stimulating qualities in mice ^[32], yet specifically blocking the OX-A receptor with a receptor specific antagonist leads to reduced feeding and obesity ^[52], ^[53]. These mechanisms behind this in mice is thought to involve proopiomelanocortin (POMC) neurones, that receive inputs from OX-A neurones in the lateral hypothalamus and promote hypophagia and weight gain by blunting POMC synthesis. These effects are supported by research that shows that it can also be reversed by peripheral intra parietal injection of an OX-A antagonist ^[54], ^[55].

Curiously, an inverse relationship seems to also exist between circulating leptin levels and OX-A, as evidenced by the observation that fasting results in the up-regulation of prepro-orexin mRNA ^[32], even in obese mice ^[56]. Additionally, several circulating hormones, such as leptin, ghrelin and glucocorticoids seem to affect OX-mediated hypothalamic circuits through synaptic rewiring ^[54], ^[55]. For more details on the involvement of the hypothalamus in obesity, recently published reviews cover the subject extensively ^[57].

3.2 Brown Adipose Tissue

Brown adipose tissue BAT is a calorie-burning fat typically associated with newborns, however its presence and metabolic activity have also been verified in adulthood ^[58]. In murine models BAT thermogenesis is activated by cold, but also a high-caloric diet and has been shown to contribute significantly to increased metabolic activity ^[59]. Rats with the gene for OX knocked-out during development display no increase in the metabolic rate of BAT after being fed a high-fat diet. They also reveal altered BAT morphology, with a higher rate of

undifferentiated preadipocytes [60]. Taken together, this suggests the involvement of OX not only in BAT activation but also in development. OX signaling in rats, from the perifornical lateral hypothalamus (PeF/LH) to the rostral raphe pallidus (rRPa) increases the excitatory drive to medullary sympathetic premotor neurones, controlling BAT sympathetic outflow and BAT thermogenesis [61].

BAT thermogenesis and its role in regulating body weight have been less studied in humans, but exposure to cold has been related to changes in BAT volume, as well as changes in its metabolic rate [58], [62]–[64]. Additionally, there is an inverse relationship between BAT volume and body weight [62] suggesting that it may play a role in the development of obesity even in humans.

However, a recent study found no difference in BAT or sympathetic nervous system activity following a two-hour cold exposure between patients with narcolepsy type-1 and healthy controls, suggesting that BAT remains functional after cold exposure even in the absence of OX neurones [65].

4. Cognitive Differences in Narcolepsy

Narcolepsy patients often report cognitive difficulties, and the most common complaint is related to subjective memory deficits [66]. Despite this, few studies have found any conclusive evidence for a genuine memory deficit; instead, results are mixed [67]–[69]. In mice, OX-A deficiencies have been shown to impact the working memory during a maze task negatively, and OX-A administration ameliorates this [70], [71]. This may be explained by the finding that OX affects long-term potentiation (LTP) in vitro, with moderate doses inhibiting LTP and sub-molar concentrations resulting in re-potentiation [72]. Ultimately, however many of these manipulations described may also influence sleep in mice and any memory deficit observed may therefore be sleep-dependent. The majority of studies on humans have reported deficits in vigilance and sustained attention in narcolepsy patients [73], [74] and cognitive dysfunction in narcolepsy is thought to be consistent with aberrant cognitive processing resources [73], [75].

OX is known to be involved in regulating attention through projections to the basal forebrain cholinergic system (BFCS). This system includes cholinergic neurones located in the nucleus basalis magnocellularis and parts of the ventral pallidum and projects to all layers of the neocortical mantle where it modulates the response of pyramidal cells to other cortical input [76]. BFCS innervation is important for cognitive function, as evidenced by pharmacological manipulations of cholinergic receptors in humans, which are known to affect attention performance [76].

This is further supported by lesion studies in animals, where the destruction of cortically projecting BFCS neurones in rats has been shown to disrupt performance in divided attention tasks [76]. This suggests that loss of OX-A may lead to instability of cortical activity, which further disrupts the efficiency of cognitive control processes. The need to sustain attention at high levels over a long period of time may then adversely interfere with working memory performance.

Default Mode Network in Attention

The default mode network (DMN) is an intrinsically activated network of functionally connected and anatomically defined set of brain regions that include the posterior cingulate cortex, precuneus, medial prefrontal cortex and angular gyrus [77], [78]. Other areas are also reported but these are the most commonly associated with the DMN. The DMN is active during periods of passive rest and "mind-wandering", this usually involves self-rumination, thinking about others and thinking about the past and future, rather than the task being performed [79]. EEG studies suggests that the DMN is reactivated within fractions of a second after the participants finished their task [80]. The DMN has also been shown to correlate negatively with other networks in the brain involved in external task such as attention and working memory and is therefore often considered a task-negative network. Recently however, this convention has been challenged by studies showing engagement of the DMN in certain external goal-oriented tasks [77].

It is known that tasks that require the continuous allocation of attention can lead to the depletion of cognitive resources [81]. As attention levels have been shown to relate to the relative level of deactivation in the DMN, deactivation within the DMN during task performance is therefore considered a good marker for deficits in sustained attention [79].

5. Methods

5.1 EEG

EEG is an electrophysiological monitoring method to record neuronal activity in the brain and it reflects the immediate mass action of neural networks from a wide range of brain systems. There is a constant flux of ions across the neuronal cell membrane, the movement of ions between neurones and their extracellular milieu is necessary in order to maintain resting potential and to propagate action potentials. Ions of similar charge repel each other and the efflux of many ions propagates leading to a wave of ion moving outward from the neurones in a process known as volume conduction ^[82].

EEG uses electrodes attached to the scalp and when the ions reach the scalp they can push or pull electrons on the metal in the electrodes. The difference in voltage between any two electrodes is then measured by a voltmeter and recorded over time giving us the EEG. EEG activity always reflects the summation of the synchronous activity of thousands or millions of neurones that have a similar spatial orientation. Neurones that do not have similar spatial orientation will not produce a detectable wave as the charges of the ions will likely cancel out each other. Additionally, voltage gradients dissipate with the square of the distance making activity from deep sources difficult to measure. Pyramidal neurones of the cortex produce a strong EEG signal because they are aligned and fire together ^[82].

EEG Microstates

It is currently thought that the momentary, global functional state of the brain is reflected by its electrical field configuration. These electrical field configurations have been shown to change discontinuously, exhibiting periods of quasi stability on the order of 100 ms before abruptly transitioning to another configuration ^[83]. These periods of quasi-stability, termed “microstates”, are thought to arise from the coordinated activity of neural assemblies originating from large areas of the cortex and have distinct scalp topographies ^[84]. Given the timescale on which EEG microstates exist, it has

been proposed that they may qualify as the basic blocks of mentation or “atoms of thought and emotion” [85].

In addition to changes to microstate topographies observed in several neurologic and neuropsychiatric diseases such as schizophrenia [86], Tourette syndrome [87], panic disorder [88], and depression [89], the temporal characteristics of EEG microstates have also been used to differentiate between diseased and normal populations.

In defining these temporal characteristics, Koenig et al. [90] proposed that the *mean duration* of a microstate reflected the stability of its underlying neural assemblies. The *frequency of occurrence* of a microstate might indicate the tendency of its underlying neural generators to be active. The *ratio of total time* covered and *global explained variance* of a given microstate are both thought to reflect the relative time coverage of its underlying neural generators compared to others.

Finally, the *transition probability* from one microstate to another can be interpreted as an encoded sequential activation of the neural assemblies that generate the microstates. Resting state EEG studies typically produce four consistently observable microstates, thought to originate from the abstract thoughts that typically arise during unstructured rest [91] and whose topographies have been arbitrarily labeled A, B, C, and D (Figure 2) [92]–[94]. These four microstates are consistently observable and can be observed even in sleep [95].

Figure 2. Topographical microstate maps.

The figure shows the resulting four microstates, which are labeled (A–D) according to previous literature. The different colours signify the different polarities. The maps are represented as seen from above (A: Anterior, P: Posterior, L: Left, R: Right) [97]

Previous studies in diseased populations have all observed changes in mean duration, frequency of occurrence, ratio of total time, and transition probability in patients relative to controls, which they interpreted as underlying changes in resting state brain dynamics characteristic of the disease (See Khanna et al. ^[96] for a more complete review of the current resting state EEG microstate literature.)

5.2 Magnetic resonance imaging

Magnetic resonance imaging (MRI) is based on the phenomenon of nuclear magnetic resonance, and involves the use of a strong magnetic field to measure the properties of the nuclear magnetic spin. This is done with the use of radio frequency pulses and the returning signal contains information about the properties of the nuclear magnetic spin, which are specific to the atom that the nuclei belong to. As the signal from the sample is measured the scanner also localises the origin of the signal in a small unit referred to as a 'voxel'. The size of the voxel can vary but is usually between 1.5-3 mm² (sometimes even up to 5 mm) and the scanner will measure each 2D-layer of voxels, so-called slices, and then combine all the slices together into one 3D-image (called volume) of the brain (Figure 3). The signal can also be acquired from the entire sample at once; so-called 3D volume imaging, advantages include improved signal-to-noise ratio while disadvantages include longer acquisition times and larger computational task for image reconstruction.

Figure 3. Schematic showing principle behind image acquisition in MRI. Voxels, ranging in size between 1.5-3 mm³ are collected in slices (thickness can vary), slices are then collected into a volume, which contains the entire sample.

The amount of time between successive pulse sequences applied to the same slice is referred to as repetition time (TR) and the time between the delivery of the RF pulse and the receipt of the echo signal is referred to as time to echo (TE) [98].

Relaxation Rates

The heart of the MRI instrument is its homogeneous magnetic field and when protons are exposed to a static magnetic field they will experience a force that causes them to align with that field. Protons can adopt only two states; either high or low energy and placing a body in a magnetic field will cause the protons in the tissue to change gradually from high energy to a low energy state until they reach equilibrium. In this condition there is a slight excess of spins in the lower energy state and it is said to be "magnetised".

Images are created through the excitation of hydrogen protons, to a high energy state, within a magnetic field using radiofrequency (RF) energy, the transition back to the lower energy "relaxed" state, after an excitation pulse can be detected as the MR-signal, and the rate at which a sample is (re-)magnetised is determined by its T1 relaxation time, which is the rate at which 63% of the magnetisation has recovered (reached equilibrium) again [98]. Typically this will be restricted to a body part of interest and the emitted signal can be located in space with the help of supplemental magnetic field gradients.

T1 and T2

T2 relaxation time, like T1 is a magnetic relaxation rate, if T2 is short then the MR-signal decreases rapidly and if it is long then the signal decreases slowly. This T2-decay is tissue specific and the R2 relaxation rate ($R2 = 1/T2$) is sensitive to the presence of metal ions, for example iron and copper, in the tissue of both normal and diseased brains. The presence of metal ions produces T2 signal decay through its paramagnetic effect on susceptibility and microscopic field gradients [99], [100].

This property can be used to image neurones containing neuromelanin, which is a dark pigment synthesised from L-DOPA as a part of the dopamine metabolism. This is because neuromelanin chelates transition metal ions, including iron, copper, and zinc making it detectable in R2 images [101]. Neuromelanin can be found in large quantities in specific nuclei of the brainstem such as the substantia nigra and the locus coeruleus (LC). Less

commonly described are the pigmented nuclei surrounding the superior cerebellar peduncle whose function to a large extent is unclear ^{[102], [103]}.

Measuring Fat and Water

Hydrogen is the simplest, most abundant element in the human body and its nuclei consist of one proton. Since the chemical structures of water and fat are unique, we would naturally expect their magnetic properties to differ. Their molecular structures give rise to two key differences in the magnetic properties of water and fat:

- T1 relaxation for fat is much shorter than those of water
- Hydrogen protons of water resonate slightly faster than those of fat. This difference in resonance frequency is known as the water-fat chemical shift.

Methods have been developed to separate water and fat signals for MR imaging and spectroscopy ^[104].

Different MRI Images

The most common MRI sequences are T1-weighted and T2-weighted scans. Using short TE and TR times produces T1-weighted images and the contrast and brightness of the image are predominately determined by T1 properties of tissue. Conversely, using longer TE and TR times produces T2-weighted images. In these images, the contrast and brightness are predominately determined by the T2 properties of tissue.

In general, looking at the CSF is a way to differentiate T1- and T2-weighted images easily, as CSF is dark on T1-weighted imaging and bright on T2-weighted imaging. Quantitative MRI (qMRI) is instead aimed at the direct measurement of physical tissue properties, such as the relaxation times, T1 and T2 and proton density (PD) ^[98].

Functional Magnetic Resonance Imaging

The advent of EEG has been described earlier in this thesis as a revolutionary method to objectively measure brain activity, other methods we have at our disposal include functional magnetic resonance imaging (fMRI) and it is the most recent and commonly used. With fMRI brain activity is measured by detecting associated changes in blood oxygenation, through measuring the so-called blood-oxygen-level-dependent (BOLD) response. The BOLD response is

caused by time-dependent changes in deoxyhemoglobin (dHb) concentration, with the blood functioning as an internal contrast agent. Blood carries hemoglobin, a protein that binds and transports oxygen molecules in the body. Hemoglobin has diamagnetic properties when it is bound to oxygen (oxyhemoglobin) conversely; it has got paramagnetic properties when not bound to oxygen (deoxyhemoglobin). In this way, deoxyhemoglobin causes the MRI signal to decrease. The BOLD-response model describes increased brain activity as the localised increase in oxygen consumption, which can then be measured because of the paramagnetic quality of deoxyhemoglobin. For more details on the mechanism of the BOLD-signal refer to these excellent reviews [105], [106].

Task-Based fMRI

In order to discern activity within specific regions in specific situations, such as in relation to fear, anxiety, or different types of mental equations, fMRI can also be used to measure activity in relation to a task. This is achieved through the use of so-called paradigms, which can consist of something as simple as a flickering checkerboard designed to evoke activation in the visual cortex. However, more complicated paradigms will require an active cognitive effort of the participant, one such example being the working memory task, which typically involves some sort of "encoding step", when the participant is typically asked to commit to memory the specific position of a letter or word within a sentence or string of letters. The response is then recorded by some software and subsequently analysed against the fluctuations of the BOLD response to evaluate increases or decreases to any specific region in relation to the task [106]-[108].

Resting State fMRI

Resting state fMRI gives information about spontaneous brain activity by measuring BOLD signal fluctuations during waking rest [109]-[112]. These resting state BOLD signal fluctuations are thought to represent spontaneous and unstructured thought processes, albeit on a much slower time scale as compared to the processing time of the brain.

Analysing the functional connectivity of these signal fluctuations using independent component analysis (ICA) or seed-voxel analysis, have yielded a predictable set of temporally stable resting state networks (RSNs) (Figure 4) [113] [114], many of which have been linked to cognitive, sensorimotor, and emotional functions [115].

Figure 4. Canonical resting state Networks. The color represents the z-statistics and can be interpreted as blood oxygen level dependent (BOLD) activation. The areas in each figure are considered to be functionally connected. The default mode network (DMN) represented in figure 13. Unmodified image from the work of Ray, McKay, Fox, Riedel, Uecker, Beckmann, Smith, Fox and Laird [116] Copyright 2018, Licensed under CC-BY 3.0, creative commons license <https://creativecommons.org/licenses/by/3.0/>

Simultaneous EEG and fMRI

The combination of EEG with its excellent temporal resolution with the superior neuroanatomical resolution of MRI has paved way for the possibility of deepening our understanding of the workings of the brain during cognition. To this end there have been many attempts to investigate the neural origins of EEG microstates further and several more recent simultaneous resting state EEG and fMRI studies have found that the occurrence of individual microstates correlated with various fMRI resting state networks [91], [117], [118].

To determine the relationship between the fMRI resting state components and the EEG microstates, the time courses for each of the four EEG microstate topographies identified from the K-means clustering analysis for each subject are first downsampled to match the temporal sampling of the fMRI data. Then, the onsets and durations of each of the four topographies are extracted for each participant individually. These onsets and durations are then used as input to the multiple regression temporal sorting algorithm in the GIFT toolbox.

The temporal sorting algorithm in GIFT [119] first convolves the input timings with the canonical hemodynamic response function to create SPM-type regressors before calculating, on an individual subject level, the slopes of the regressors between the time courses of each of the EEG microstates and the time courses of each of the RSNs of interest.

These slopes are then averaged for each RSN and EEG microstate pairing for each group separately, converted to standardised Z-scores, and displayed as a heat map. Only those pairings with a Z-score of at least one should be considered for interpretation.

Measuring GABA and Glutamate

Proton Magnetic resonance spectroscopy (H-MRS) can be used to non-invasively measure concentrations of neurotransmitters and detect biochemical changes in the brain, in-vivo. Just like MRI it uses pulse signal to acquire signal from several different molecules, but it generates a spectrum instead of an image. γ -Amino-Butyric Acid (GABA) is the main inhibitory neurotransmitter in the human brain and is synthesised from Glutamate, the main excitatory neurotransmitter [120], [121].

Both GABA and Glutamate can be measured with H-MRS and narcolepsy patients have been found to have increased concentrations of GABA in the medial prefrontal cortex. In addition to this GABA can also be related to the BOLD signal using H-MRS ^[122] and resting-state GABA concentrations in the anterior cingulate cortex (ACC) have been found to correspond to task-related negative BOLD responses in the same region ^[123].

Plasma concentrations of a positive allosteric modulator of GABA were also found to be inversely correlated with BOLD responses in the ACC ^[123] and taken together this suggests that GABA may mediate negative BOLD responses, at least in the ACC. It's further been shown that endogenous levels of GABA correlate inversely with measured BOLD responses in the DMN during the performance of a working memory task ^{[123], [124]}.

6. Aims

Narcolepsy is a chronic, debilitating sleep disorder characterised by excessive sleepiness and sleep attacks, and patients often suffer other non-sleep related symptoms as well; such as cognitive difficulties and obesity.

Disease prevalence is 25-50 per 100000 people, however, because narcolepsy often goes unrecognised or misdiagnosed, determining its true frequency in the general population is difficult. Since the aetiology of canine narcolepsy was discovered in 1999 narcolepsy research has made some great strides, most notably in finding that it may have an auto-immunological cause. However, few studies have studied human patients and even fewer of those using imaging technology.

The overarching aim of this thesis is to investigate the mechanisms and aetiology behind the symptoms in narcolepsy through the application of different magnetic resonance imaging techniques and EEG.

This can be further related more specifically, where the aims are threefold and as follows:

- Investigate brain function in narcolepsy (*Papers I & II*)
- Investigate brain stem structural differences in narcolepsy (*Paper III*)
- Investigate if the loss of Orexin is related to brown adipose tissue fat distribution (*Paper IV*)

7. Results

7.1 Brain Function in Narcolepsy

The first aim of this thesis was to investigate neural correlates of cognitive deficits previously reported in narcolepsy. With respect to this, different brain imaging modalities were used in order to specifically investigate brain function related to working memory and sustained attention.

Microstates-Resting State fMRI

Resting state fMRI networks give information about what brain networks are engaged during unstructured rest. In paper I we aimed to further explore resting state fMRI by combining it with EEG, taking advantage of both the superior neuroanatomical localisation of fMRI and the millisecond-range temporal resolution of EEG. Much like the resting state networks, EEG microstates are thought to represent network activity, albeit at a much higher temporal resolution. Our analysis resulted in four microstates topographically similar to those previously described in the literature (*Figure 2*). Analysis of their dynamics revealed that narcolepsy patients spent less of the scan-time in microstate A (and more in "B" and "C"), suggesting an underlying instability of this microstate, and more stability in "B" and "C" (*Figure 5*).

Temporal sorting of the RSNs based on the time-course of the EEG resulted in 15 networks that correlated with the time-course of the EEG (*Figures 6 & 7*), and where microstate A correlated most strongly with components comprised of the anterior and posterior aspects of the DMN in both patients and controls (*Figure 6*). The combined result of the microstate-fMRI analysis therefore indicates that narcolepsy patients may engage the posterior and anterior aspects of the DMN less than do the healthy controls.

In addition to the DMN, microstate A also correlated with the primary visual cortex component, in narcolepsy patients, suggesting that multiple neural networks may be contributing to the electrical neural field generation for microstate A in the narcolepsy patients, indicating abnormal resting state brain dynamics.

Figure 5. Results from the electroencephalography (EEG) microstates analysis. The figure shows (A) mean duration of each microstate (in ms), (B) mean global explained variance (GEV), and (C) ratio of total time covered for each microstate. The error bars represent standard deviation.

** Indicates a significant post hoc difference. °° Indicates a trend-level post hoc difference.

Poor sleep habits are known to affect cognitive function, therefore sleep-behaviour data was also collected using actigraphy. Actigraphy is a commonly used method to measure sleep-behaviour, in which a small unit (called actigraphy sensor) is worn on the arm for a week to record gross motor activity. In paper I, we report that narcolepsy patients had significantly lower sleep efficacy (narcolepsy 63%; controls 82%, $p < 0.01$) in the week following up to the data collection.

We also attempted to control for sleep, by sleep scoring the EEG and found that there were no significant group differences in sleep between narcolepsy patients and healthy controls during MRI scanning.

Figure 6. Temporal correlation of EEG microstates and functional magnetic resonance imaging (fMRI) RSNs. Results of the temporal sorting of the RSNs using the time courses of the four EEG microstates. Results are stratified in terms of microstate and study group, with results for the narcolepsy patients and controls displayed separately. The temporal sorting regression coefficients are displayed in terms of Z-scores. *Indicates a temporal correlation with a Z-score >1.

Figure 7. Resting State Networks (RSNs) Representative slices for each of the 15 RSNs that correlated with the microstates after the temporal sorting. The functional connectivity maps are rendered as 1-sample t-tests ($p < 0.05$, family wise error (FWE) corrected for comparing across the whole brain) across the entire study sample of narcolepsy patients and healthy controls.

Working Memory and Sustained Attention

Tasks, which require the continuous maintenance of attention, can lead to a depletion of cognitive resources and relative deactivation of the DMN can be used to indicate current levels of attention. A decrease in relative deactivation is typically indicative of deficits in sustained attention. Furthermore, activation in left-middle-frontal-gyrus (LMFG) is related to working memory ability. In paper II we found, that narcolepsy patients had a greater deactivation in the DMN during the performance of the encoding and retrieval parts of a verbal working memory task, compared to healthy controls (*Figure 8*). This deactivation was not seen together with any decrease in LMFG activation and we found no differences in task performance between narcolepsy patients and the healthy controls.

Additionally, even though no significantly reduced activity was found in the LMFG, there was a positive correlation with increased deactivation in the DMN during the encoding of sentences (*Figure 9A*). Encoding of sentences in a verbal working memory paradigm that typically requires sustained attention.

The results suggest that cognitive resources may be overly focused on maintaining sufficient levels of attention.

Figure 8. Representative axial slices of group-level fMRI activation during the working memory task across all subjects. A Encoding of sentences. B Recognition of words. All activation maps were thresholded at $p < 0.05$, using Family Wise Error correction for comparing across all voxels in the brain. Colour bars are scaled in terms of t-statistic. Slices were created using Mango (<http://ric.uthscsa.edu/mango/> Jack L. Lancaster and Michael J. Martinez)

Figure 9. Correlation analysis comparing activation levels in left middle frontal gyrus (LMFG) and DMN. *A* Encoding of sentences. *B* Recognition of words. *C* Parametric effect of load during encoding of sentences. *D*. Parametric effect of load during recognition of words. Dashed lines indicate best linear fit of all data across both groups. For illustrative purposes, narcolepsy data points are indicated by light grey diamonds and healthy controls by dark grey squares

When looking at task load, i.e. the effect of increasing difficulty of the task, we found increased activation in the DMN of narcolepsy patients for the encoding of sentences (*Figure 10C*). Typically an increase in workload is associated with higher levels of deactivation in the DMN [79] as higher effort should require more allocation of cognitive resources to task positive brain regions.

This finding may show that narcolepsy patients are making use of this network to perform the task, or it could represent a release of resources that would otherwise be used to suppress activity in the DMN, in favour of allocation towards task positive related brain regions.

Decreased concentrations of GABA+ (GABA plus macromolecular contamination in the MRS signal [125]), together with increased levels of Glutamate in the medial prefrontal cortex (mPFC), correlated with increased levels of deactivation during the encoding of sentences in narcolepsy patients (*Figure 11*).

Figure 10. Between-group comparisons of activation in the default mode network (DMN). **A** Encoding of sentences. **B** Recognition of words. **C.** Parametric effect of load during recognition of words. Activation displayed as estimated marginal means (corrected for age, gender, and number of missed trials) of beta values, with healthy controls shown in dark grey and narcolepsy patients in light grey. Error bars are given in terms of standard error

Figure 11. Results from correlation analysis comparing GABA+ and Glutamate concentrations to BOLD activity levels in medial prefrontal cortex (mPFC) during the encoding of sentences. **A.** GABA+ with deactivation in medial prefrontal cortex (mPFC). **B.** Glutamate with deactivation in mPFC. Dashed lines indicate best linear fit, and narcolepsy data points are indicated by light gray diamonds and healthy controls by dark grey squares

The results point towards an active suppression or some form of metabolic dysregulation of at least the anterior portion of the default mode network. The opposite was observed for the narcolepsy patients.

7.2 Brain Structure and Connectivity

In paper III, we identified a small area in the brain stem's rostral reticular formation near the superior cerebellar peduncle, which exhibited lower R2 levels in narcolepsy patients. We suggested that it is the LC due to the coordinates of our identified area falling near previously described LC locations ^{[126][127]} (Figure 12). In addition to this LC contains neuromelanin, a dark pigment synthesized from L-DOPA as a part of the dopamine metabolism. R2 relaxation rate [$R2 = 1/T2$] can be used to detect neuromelanin-containing neurones due to its sensitivity to metal ions ^[128].

Figure 12. Lower R2 values in narcolepsy. The figures show statistically significant R2 differences between narcolepsy patients and healthy controls in sagittal (left image), axial (right upper image) and coronal (right lower image) planes. Narcolepsy patients had significantly lower R2 than controls in a bilateral area in the rostral reticular formation adjacent to the superior cerebellar peduncle, peak coordinates = 7 -23 -15. The images are shown with an uncorrected threshold of $p = 0.001$. A = anterior; P = posterior; L = left; R = right; S = superior.

Sleep efficacy is a measure of time spent sleeping as a fraction of time spent lying down and low sleep efficacy is often due to experiencing difficulties falling asleep but could also reflect fragmented night sleep.

Kumar *et al* showed that R2 in regional brain sites changes as part of normal ageing, even in healthy people and that this change can be detected even in young people [129].

We applied the value for the slope from the correlation published for the cerebellar peduncle to correct R2 values for the differences in age between all of our participants.

After correcting the R2 values for participants age, we found a significant correlation with sleep efficacy, $r = 0.57$; $p = 0.0014$, but not to total sleep duration, $p = 0.24$, or cataplexy, $p = 0.06$ (Figure 13A).

There was a seeming trend correlation between R2 and cataplexy, however it was clearly driven by group differences (Figure 13B). No significant correlations were found between R2 and any of the clinical measures after separating narcolepsy and healthy controls into two groups, $p > 0.1$. However, as seen in Figure 10A, the regression line of R2 and sleep efficacy in narcolepsy with slope = 10.7 follows the regression line for all participants, slope = 10.5.

Figure 13. Results from correlation between R2 and clinical measures. The figure shows the relations between mean R2 values extracted from the deviant area in the rostral reticular formation (Figure 10) and sleep efficacy (A), total sleep duration (B), and cataplexy (C) in narcolepsy patients and healthy controls. R2 is presented as age corrected R2 to account for the differences in age between all participants. The black lines represent the linear regression for all participants, the dotted lines represent the linear regression for narcolepsy (red) and healthy controls (blue), respectively. ** = $p < 0.01$; ns = not significant

Connectivity

We found that the R2 deviant area in the rostral reticular formation was functionally connected to several regions related to OX signaling e.g., the hypothalamus, ventral tegmental area (VTA), nucleus accumbens (NAc), amygdala (AMY), hippocampus (HC), pallidum, and the thalamus, $pFDR < 0.05$ (Figure 14). We also observed significant connections to the parahippocampal gyrus, the lingual gyrus, the fusiform gyrus, the posterior cingulate cortex, and the cerebellum. Detailed reports of functional connectivity results can be found in paper III. This supports the involvement of parts of the reticular formation and the reticular activating system, and further supports that the area observed in our sample is the LC.

Figure 14. Functional connectivity of the rostral reticular formation. The figure shows functional connectivity of the R2 deviant area in the rostral reticular formation adjacent to the superior cerebellar peduncle in nine sagittal slices representing all participants.

In addition to the LC projections from the hypothalamus, which are related to sleep stability, LC is functionally connected via hypothalamic projections to the VTA, AMY and NAc^[130], areas involved in wakefulness and cataplexy. We found no group differences in functional connectivity between narcolepsy patients and healthy controls.

7.3 Orexin and brown adipose tissue

Obesity is common in narcolepsy, the extent to which weight gain in narcolepsy is caused by lack of sleep or some other disease-specific mechanism has still not been fully determined.

In paper IV, we examined the possibility that lack of OX could affect BAT. We expected to find lower volumes of BAT in narcolepsy patients, compared to healthy controls, however we saw no significant group differences (*Figure 15*). We did however see that narcolepsy patients had less visceral fat, relative to their total subcutaneous fat. We had not collected OX samples from the CSF of the participants, however we did have samples collected from the patients at the time of diagnosis. We found no correlation between the OX concentrations in CSF and any of the fat measures.

We concluded that lack of OX has no effect on BAT volume in narcolepsy.

Figure 15. Visceral adipose tissue ratio results. Visceral adipose tissue ratio (VATR) was lower in narcolepsy patients. VAT= Visceral adipose tissue. VATR= visceral adipose tissue ratio, VAT/VAT+ASAT. ASAT= abdominal subcutaneous adipose tissue

8. Discussion

8.1 Cognitive Complaints in Narcolepsy

Even though most studies involving humans have not been able to find evidence of any true memory deficit in narcolepsy, many patients report complaints related to memory. In this thesis, I present evidence which weighs in favour of the theory that narcolepsy patients do not suffer from deficits of working memory, but rather that their perceived memory struggle may have its seat in impairment of the networks involved in the maintenance of sustained attention.

In paper I resting state fMRI coupled with EEG microstate analysis revealed that narcolepsy patients differed significantly in dynamics of the DMN. This was seen both through aberrant microstate dynamics that were correlated with the DMN (extracted from resting state fMRI), but also when inspecting the resting state networks where the DMN was found to include aspects of the primary visual cortex. Considering how microstates are thought to originate from the same neural originators as is seen in fMRI, albeit at a higher time resolution, the results suggest the involvement of multiple neural networks in the electrical neural field generation for that microstate.

Specifically investigating areas related to working memory function (the LMFG), as well as sustained attention (the DMN) in paper II revealed no evidence of a true working memory deficit, as evidenced by the lack of altered activation in the LMFG. In contrast, a greater deactivation was seen in the DMN of narcolepsy patients during the parts of the task, which related to sustained attention. Surprisingly, deactivation in the DMN correlated with decreased GABA⁺ and increased Glutamate concentration in the mPFC.

GABA is typically involved in suppressing the DMN during exogenous cognitive tasks, even if the results showed only trend level significance, our finding of the contrary suggests a disruption to the normal GABA suppression at least in the anterior portion of the DMN. It may be that Glutamate is involved in actively suppressing the DMN instead, given that there is a positive correlation between increased Glutamate concentrations in the mPFC and DMN deactivation. All of this taken together suggest that

narcolepsy patients have a deficit in DMN caused by aberrant GABA and Glutamate signaling, ultimately resulting in a misallocation of cognitive resources.

This could potentially point to future non-pharmaceutical therapeutic interventions involving the DMN.

8.2 Obesity and Sleep

The topic of BAT has been, what is often referred to in popular science communication as, *trending* over the past few years. Most commonly, interests relate to its therapeutic potential as an "anti-obesity" tissue, due to its metabolic qualities. OX has been shown to have an effect on the activation of BAT, at least in murine models where knockout animals also show morphological differences in BAT directly related to reduced metabolic activity [60].

Unlike in mice, where weight gain in narcolepsy is seen even in the face of hypophagia, there is still no consensus as to whether this is true in humans as well. While most narcolepsy patients do gain weight, the correlation between narcolepsy and obesity is weaker than in murine models. In paper IV no difference was found in BAT volume between narcolepsy-type 1 patients and their healthy peers, leading us to draw the conclusion that loss of OX, as seen in narcolepsy type-1 and any subsequent weight gain, may not be related to differences in BAT volume.

Sleep deprivation is known to cause weight gain even in healthy people [131], [132] in paper III we found a structural anomaly in the brainstem which correlated strongly with sleep efficacy, suggesting that the narcolepsy patients may be experiencing weight gain due to metabolic alterations resulting from sleep deprivation. However, at least one study suggests that weight gain is independent of sleep disturbances in narcolepsy and other studies have observed lower metabolism in narcolepsy [49],[133]. The lack of any measure for metabolic status, which could have helped determine the potential role of sleep should be noted and considered for any future studies.

The lack of a measure of BAT activity, together with OX concentrations was a clear disadvantage to this study. The finding that the narcolepsy patients had a lower relative visceral adipose tissue when compared to the controls was curious.

A clinical study by Porter S.A. et al. (2009) compared the metabolic and cardiovascular disease risk between people with either high or low VAT in conjunction with high or low volumes of subcutaneous adipose tissue. They found that disease risk for those who were in the highest percentile for VAT was inversely related to the volume of subcutaneous adipose tissue. For those in the lower percentiles, this relationship was not seen. They conclude that subcutaneous adipose tissue may potentially have a protective effect in some individuals, specifically in those with a higher volume of VAT^[134]. Though this has not been further studied in humans, the conclusions of the authors are supported by murine model studies, where surgical removal of subcutaneous adipose tissue from high VAT animals caused a decrease in glucose tolerance and insulin sensitivity ^[135]. Reversely, transplant of subcutaneous tissue into high VAT animals resulted in alleviation of diet induced metabolic disease ^[135], ^[136]. We might speculate that the increase in subcutaneous adipose tissue observed in narcolepsy patients is in response to an excess of fatty acids in the vascular compartment, and may have a protective effect.

Any interpretation as to the implications of this can only be cautiously inferred from previously published studies, which show that there may be some protective effect of subcutaneous adipose tissue in people, particularly in those with a large volume of visceral fat.

8.3 Potential Targets for Treatment and Biomarkers

Brainstem

OX-neurons in the hypothalamus project widely across the brain and while it is well known that many of the symptoms in narcolepsy are related their loss, no evidence of macroscopic lesions have been observed in sporadic narcolepsy. In paper III, we report that adolescent narcolepsy patients have structural deviations, as measured by R2-level differences, in the rostral reticular formation of the brain stem.

We suggest that these observed differences could be due to lower levels of neuromelanin in the LC of narcolepsy patients. Several of the findings point specifically to the LC as the identified area:

- R2 is sensitive to metal ions in tissue; LC contains neuromelanin, which is known to chelate metal ions.

- The R2 relaxation rate in this area was related to sleep efficacy and the LC is important for maintaining sleep stability.
- This area is also shown in paper III to be functionally connected to OX signaling pathways, including the hypothalamus.

Neuromelanin deviations have also been reported in the LC of patients suffering from Parkinson's disease, Alzheimer's, and Dementia [128] and has been proposed as a biomarker. However unlike narcolepsy these are all neurodegenerative diseases and the extent to which it can be used as a biomarker in narcolepsy remains to be evaluated. In addition to this, this study had a relatively small size and our patient group had all but one been diagnosed following the Pandemrix vaccination. Considering the differences in symptom severity seen in this patient group, it is possible that the area observed could be specific to Pandemrix related narcolepsy.

This finding does however provides new insights into the pathophysiology of narcolepsy beyond the well-known depletion of OX producing neurones in the hypothalamus, which has the potential to provide new targets for drug therapy.

Default Mode Network

Different interventions designed to affect the activity or connectivity of the DMN is a new potential method of treating neurological disorders (e.g. post traumatic stress disorder and obsessive-compulsive disorder) [112][137], [138]. Behavioural training studies have also demonstrated the potential of modulating DMN activity through real-time fMRI neurofeedback training [139]. The extent to which any such intervention would be meaningful in narcolepsy is highly speculative and future studies on larger populations need first to confirm DMN dysfunction in narcolepsy. However, it might be a potential target for future studies at least in addressing cognitive deficits in narcolepsy.

Electroencephalography

A drawback with EEG in general is the lack of reliable spatial information and previous EEG analysis methods required the reliance of a reference electrode, which makes cross-study comparisons difficult. The introduction of the microstate analysis allows for reference free analysis, which subsequently facilitates the use of the method for e.g. EEG biomarkers. Compared with fMRI, EEG is inexpensive and portable, making it accessible even for remote areas and smaller budgets. Combining fMRI and microstates holds the potential for characterising the microstates as correlating to specific brain network which has both clinical and research value. Limitations with the microstate analysis include the diverse methodology employed by many studies, with some using spatial correlation ^{[91], [118], [141]} and at least one other using temporal correlation ^[140] and no consistent relationships between individual microstates and fMRI-derived resting state networks yet exist.

8.4 Conclusion

The Aim of this thesis was to investigate mechanisms and aetiology behind the symptoms in narcolepsy through the application of different imaging techniques. I present in this thesis evidence supporting that the complaints about subjective memory deficits in narcolepsy are related to a misallocation of cognitive resources.

I further describe how this has its seat in defective DMN activation, possibly involving alterations to GABA and Glutamate signaling. In addition to this, I present our findings of a structural deviation in an area of the brainstem previously not described in the aetiology of narcolepsy. This finding may have implications for further understanding the aetiology of the disease and the specific neuronal populations involved.

In addition to this, I show evidence from adipose tissue measurements in specific compartments, confirming that weight gain in narcolepsy is characterised by centrally located weight gain and may be specifically related to OX changes, but maybe not BAT volume.

Finally, I propose Three new targets for evaluation as biomarkers or potential treatment.

References

- [1] W. G. LENNOX, "THOMAS WILLIS ON NARCOLEPSY," *Arch. Neurol. Psychiatry*, vol. 41, no. 2, p. 348, Feb. 1939.
- [2] C. H. Schenck, C. L. Bassetti, I. Arnulf, and E. Mignot, "English translations of the first clinical reports on narcolepsy and cataplexy by Westphal and Gélinau in the late 19th century, with commentary," *Journal of Clinical Sleep Medicine*. 2007.
- [3] L. Löwenfeld, "Über Narkolepsie," *Munch. Med. Wochenschr*, vol. 49, pp. 1041–1045, 1902.
- [4] S. Wilson, "The narcolepsies," *Annu. Congr. Assoc. Phys*, vol. June 3, pp. 63–109, 1927.
- [5] R. E. Yoss and D. D. Daly, "Criteria for the diagnosis of the narcoleptic syndrome.," *Proc. Staff Meet. Mayo Clin.*, 1957.
- [6] R. T. Ravenholt and W. H. Foege, "1918 INFLUENZA, ENCEPHALITIS LETHARGICA, PARKINSONISM," *Lancet*, 1982.
- [7] M. S. Dickman, "Von Economo encephalitis," *Archives of Neurology*. 2001.
- [8] K. Susmáková, "Human Sleep and Sleep EEG," *Meas. Sci. Rev.*, 2004.
- [9] R. B. Berry, R. Brooks, C. E. Gamaldo, S. M. Harding, C. L. Marcus, and B. V. Vaughn, "The AASM Manual for the Scoring of Sleep and Associated Events Version 2.5," 2018.
- [10] C. Ruoff and D. Rye, "The ICSD-3 and DSM-5 guidelines for diagnosing narcolepsy: clinical relevance and practicality," *Current Medical Research and Opinion*. 2016.
- [11] A. P. Association, *DSM 5*. 2013.
- [12] Y. Dauvilliers, I. Arnulf, and E. Mignot, "Narcolepsy with cataplexy," *Lancet*, 1980.
- [13] A. M. Winstone et al., "Clinical features of narcolepsy in children vaccinated with AS03 adjuvanted pandemic A/H1N1 2009 influenza vaccine in England ICSD International Classification of Sleep Disorders HES Hospital Episode Statistics HLA Human leukocyte antigen NHS National Hea."
- [14] American Academy of Sleep Medicine, "International classification of sleep disorders—third edition (ICSD-3)," *AASM Resour. Libr.*, 2014.
- [15] A. Crocker et al., "Concomitant loss of dynorphin, NARP, and orexin in narcolepsy.," *Neurology*, vol. 65, no. 8, pp. 1184–8, Oct. 2005.

- [16] T. C. Thannickal et al., "Reduced number of hypocretin neurons in human narcolepsy," *Neuron*, vol. 27, no. 3, pp. 469–74, Sep. 2000.
- [17] C. Peyron et al., "A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains," *Nat. Med.*, vol. 6, no. 9, pp. 991–997, Sep. 2000.
- [18] L. de Lecea et al., "The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 95, no. 1, pp. 322–7, Jan. 1998.
- [19] T. Sakurai et al., "Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior," *Cell*, vol. 92, no. 4, pp. 573–85, Feb. 1998.
- [20] M. G. Lee, O. K. Hassani, and B. E. Jones, "Discharge of Identified Orexin/Hypocretin Neurons across the Sleep-Waking Cycle," *J. Neurosci.*, vol. 25, no. 28, pp. 6716–6720, Jul. 2005.
- [21] A. R. Adamantidis, F. Zhang, A. M. Aravanis, K. Deisseroth, and L. de Lecea, "Neural substrates of awakening probed with optogenetic control of hypocretin neurons," *Nature*, vol. 450, no. 7168, pp. 420–424, Nov. 2007.
- [22] R. M. Chemelli et al., "Narcolepsy in orexin Knockout Mice," *Cell*, vol. 98, no. 4, pp. 437–451, Aug. 1999.
- [23] L. Lin et al., "The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene," *Cell*, 1999.
- [24] S. Nishino, B. Ripley, S. Overeem, G. J. Lammers, and E. Mignot, "Hypocretin (orexin) deficiency in human narcolepsy," *Lancet*, 2000.
- [25] T. E. Scammell, A. Crocker, S. McCormack, M. Yanagisawa, T. Sakurai, and T. Mochizuki, "Behavioral state instability in orexin knockout mice," in *Sleep and Biological Rhythms*, 2004.
- [26] K. Sasaki, M. Suzuki, M. Mieda, N. Tsujino, B. Roth, and T. Sakurai, "Pharmacogenetic modulation of orexin neurons alters sleep/wakefulness states in mice," *PLoS One*, 2011.
- [27] A. F. Branch et al., "Progressive Loss of the Orexin Neurons Reveals Dual Effects on Wakefulness," *Sleep*, vol. 39, no. 2, pp. 369–377, Feb. 2016.
- [28] E. Hasegawa, M. Yanagisawa, T. Sakurai, and M. Mieda, "Orexin neurons suppress narcolepsy via 2 distinct efferent pathways," *J. Clin. Invest.*, vol. 124, no. 2, pp. 604–616, Feb. 2014.
- [29] M. E. Carter, J. Brill, P. Bonnavion, J. R. Huguenard, R. Huerta, and L. de Lecea, "Mechanism for Hypocretin-mediated sleep-to-wake transitions," *Proc. Natl. Acad. Sci.*, vol. 109, no. 39, pp. E2635–E2644, Sep. 2012.
- [30] B. Y. Mileykovskiy, L. I. Kiyashchenko, and J. M. Siegel, "Behavioral Correlates of Activity in Identified Hypocretin/Orexin Neurons," *Neuron*, vol. 46, no. 5, pp. 787–798, Jun. 2005.

- [31] H. Hor et al., "Genome-wide association study identifies new HLA class II haplotypes strongly protective against narcolepsy," *Nature Genetics*. 2010.
- [32] J. M. Zeitzer, S. Nishino, and E. Mignot, "The neurobiology of hypocretins (orexins), narcolepsy and related therapeutic interventions," *Trends in Pharmacological Sciences*. 2006.
- [33] E. Mignot et al., "Complex HLA-DR and -DQ Interactions Confer Risk of Narcolepsy-Cataplexy in Three Ethnic Groups," *Am. J. Hum. Genet.*, 2001.
- [34] M. Partinen, B. R. Kornum, G. Plazzi, P. Jennum, I. Julkunen, and O. Vaarala, "Narcolepsy as an autoimmune disease: The role of H1N1 infection and vaccination," *The Lancet Neurology*. 2014.
- [35] E. Mignot, tR Hayduk, and F. C. Grumet, "Narcolepsy HLA DQB 1 *0602 is Associated With Cataplexy In 509 Narcoleptic Patients," *Sleep*, 2018.
- [36] J. Hallmayer et al., "Narcolepsy is strongly associated with the T-cell receptor alpha locus," *Nat. Genet.*, 2009.
- [37] H. Petrie, "T cell receptor gene recombination patterns and mechanisms: cell death, rescue, and T cell production," *J. Exp. Med.*, 1995.
- [38] A. K. Singh, J. Mahlios, and E. Mignot, "Genetic association, seasonal infections and autoimmune basis of narcolepsy," *J. Autoimmun.*, 2013.
- [39] T. J. Dye, N. Gurbani, and N. Simakajornboon, "Epidemiology and Pathophysiology of Childhood Narcolepsy," *Paediatric Respiratory Reviews*. 2018.
- [40] B. R. Kornum et al., "Narcolepsy," *Nat. Rev. Dis. Prim.*, vol. 3, p. 16100, Feb. 2017.
- [41] S. S. Ahmed, P. H. Schur, N. E. MacDonald, and L. Steinman, "Narcolepsy, 2009 A(H1N1) pandemic influenza, and pandemic influenza vaccinations: What is known and unknown about the neurological disorder, the role for autoimmunity, and vaccine adjuvants," *Journal of Autoimmunity*. 2014.
- [42] H. Nohynek et al., "AS03 adjuvanted AH1N1 vaccine associated with an abrupt increase in the incidence of childhood narcolepsy in Finland," *PLoS One*, 2012.
- [43] A. Aran et al., "Elevated anti-streptococcal antibodies in patients with recent narcolepsy onset," *Sleep*, 2009.
- [44] American Psychiatric Association, "Highlights of Changes from DSM-IV-TR to DSM-5," *Am. Psychiatr. Assoc. Washington, ...*, 2013.
- [45] D. B. Rye, B. Dihenia, J. D. Weissman, C. M. Epstein, and D. L. Bliwise, "Presentation of narcolepsy after 40," *Neurology*, 1998.
- [46] O. Andlauer et al., "Predictors of Hypocretin (Orexin) Deficiency in Narcolepsy Without Cataplexy," *Sleep*, 2012.

- [47] T. C. Thannickal, R. Nienhuis, and J. M. Siegel, "Localized loss of hypocretin (orexin) cells in narcolepsy without cataplexy," *Sleep*, 2009.
- [48] E. Mignot et al., "The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias," *Arch. Neurol.*, 2002.
- [49] S. W. Kok et al., "Hypocretin deficiency in narcoleptic humans is associated with abdominal obesity," *Obes. Res.*, vol. 11, no. 9, pp. 1147–1154, 2003.
- [50] G. J. Lammers, H. Pijl, J. Iestra, J. A. E. Langius, G. Buunk, and A. E. Meinders, "Spontaneous food choice in narcolepsy," *Sleep*, 1996.
- [51] J. Hara et al., "Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity," *Neuron*, 2001.
- [52] T. Shiraishi, Y. Oomura, K. Sasaki, and M. J. Wayner, "Effects of leptin and orexin-A on food intake and feeding related hypothalamic neurons," *Physiol. Behav.*, 2000.
- [53] A. C. Haynes et al., "A selective orexin-1 receptor antagonist reduces food consumption in male and female rats," in *Regulatory Peptides*, 2000.
- [54] G. Morello et al., "Orexin-A represses satiety-inducing POMC neurons and contributes to obesity via stimulation of endocannabinoid signaling," *Proc. Natl. Acad. Sci.*, 2016.
- [55] L. Cristino et al., "Obesity-driven synaptic remodeling affects endocannabinoid control of orexinergic neurons," *Proc. Natl. Acad. Sci.*, 2013.
- [56] A. Yamanaka et al., "Hypothalamic orexin neurons regulate arousal according to energy balance in mice," *Neuron*, 2003.
- [57] R. Imperatore, L. Palomba, and L. Cristino, "Role of Orexin-A in Hypertension and Obesity," *Current hypertension reports*. 2017.
- [58] K. A. Virtanen et al., "Functional Brown Adipose Tissue in Healthy Adults," *N. Engl. J. Med.*, 2009.
- [59] M. A. Griggio, "The participation of shivering and nonshivering thermogenesis in warm and cold-acclimated rats," *Comp. Biochem. Physiol. -- Part A Physiol.*, 1982.
- [60] D. Sellayah, P. Bharaj, and D. Sikder, "Orexin is required for brown adipose tissue development, differentiation, and function," *Cell Metab.*, 2011.
- [61] S. F. Morrison, C. J. Madden, and D. Tupone, "An orexinergic projection from perifornical hypothalamus to raphe pallidus increases rat brown adipose tissue thermogenesis," *Adipocyte*, 2012.
- [62] W. D. van Marken Lichtenbelt et al., "Cold-Activated Brown Adipose Tissue in Healthy Men," *N. Engl. J. Med.*, 2009.
- [63] M. Borga et al., "Brown adipose tissue in humans: Detection and functional analysis using PET (positron emission tomography), MRI (magnetic resonance

- imaging), and DECT (dual energy computed tomography)," *Methods Enzymol.*, 2014.
- [64] T. Romu et al., "Characterization of brown adipose tissue by water-fat separated magnetic resonance imaging," *J. Magn. Reson. Imaging*, 2015.
 - [65] L. H. Enevoldsen et al., "Functional brown adipose tissue and sympathetic activity after cold exposure in humans with type 1 narcolepsy," *Sleep*, 2018.
 - [66] R. Broughton, Q. Ghanem, Y. Hishikawa, Y. Sugita, S. Nevsimalova, and B. Roth, "Life Effects of Narcolepsy in 180 Patients from North America, Asia and Europe Compared to Matched Controls," *Can. J. Neurol. Sci. / J. Can. des Sci. Neurol.*, 1981.
 - [67] M. Aguirre, R. Broughton, and D. Stuss, "Does memory impairment exist in Narcolepsy-Cataplexy?," *J. Clin. Exp. Neuropsychol.*, 1985.
 - [68] A. E. Rogers and R. S. Rosenberg, "Tests of memory in narcoleptics," *Sleep*, 1990.
 - [69] B. Hood and D. Bruck, "Metamemory in narcolepsy," *J. Sleep Res.*, 1997.
 - [70] L. Yang et al., "Hypocretin/orexin neurons contribute to hippocampus-dependent social memory and synaptic plasticity in mice," *Ann. Intern. Med.*, 2013.
 - [71] N. Nevárez and L. de Lecea, "Recent advances in understanding the roles of hypocretin/orexin in arousal, affect, and motivation," *F1000Research*, vol. 7, p. 1421, Sep. 2018.
 - [72] M. J. Wayner, D. L. Armstrong, C. F. Phelix, and Y. Oomura, "Orexin-A (Hypocretin-1) and leptin enhance LTP in the dentate gyrus of rats in vivo," *Peptides*, 2004.
 - [73] L. Zamarian et al., "Subjective deficits of attention, cognition and depression in patients with narcolepsy," *Sleep Med.*, 2015.
 - [74] M. Rieger, G. Mayer, and S. Gauggel, "Attention deficits in patients with narcolepsy," *Sleep*, 2003.
 - [75] A. Naumann, C. Bellebaum, and I. Daum, "Cognitive deficits in narcolepsy," *J. Sleep Res.*, 2006.
 - [76] J. Fadel and J. A. Burk, "Orexin/hypocretin modulation of the basal forebrain cholinergic system: Role in attention," *Brain Res.*, 2010.
 - [77] J. R. Andrews-Hanna, J. Smallwood, and R. N. Spreng, "The default network and self-generated thought: Component processes, dynamic control, and clinical relevance," *Ann. N. Y. Acad. Sci.*, 2014.
 - [78] R. L. Buckner, J. R. Andrews-Hanna, and D. L. Schacter, "The brain's default network: Anatomy, function, and relevance to disease," *Annals of the New York Academy of Sciences*. 2008.

- [79] D. H. Weissman, K. C. Roberts, K. M. Visscher, and M. G. Woldorff, "The neural bases of momentary lapses in attention," *Nat. Neurosci.*, 2006.
- [80] M. Dastjerdi et al., "Differential electrophysiological response during rest, self-referential, and non-self-referential tasks in human posteromedial cortex," *Proc. Natl. Acad. Sci.*, 2011.
- [81] J. S. Warm, R. Parasuraman, and G. Matthews, "Vigilance Requires Hard Mental Work and Is Stressful," *Hum. Factors J. Hum. Factors Ergon. Soc.*, 2008.
- [82] P. Olejniczak, "Neurophysiologic basis of EEG," in *Journal of Clinical Neurophysiology*, 2006.
- [83] D. Lehmann, H. Ozaki, and I. Pal, "EEG alpha map series: brain micro-states by space-oriented adaptive segmentation," *Electroencephalogr. Clin. Neurophysiol.*, 1987.
- [84] G. Roks et al., "EEG microstate analysis (1) ," *Clin. Neurophysiol.*, 2014.
- [85] D. Lehmann and C. M. Michel, "Eeg-defined functional microstates as basic building blocks of mental processes," *Clin. Neurophysiol.*, vol. 122, no. 6, pp. 1073–1074, 2011.
- [86] C. Andreou et al., "Resting-state connectivity in the prodromal phase of schizophrenia: Insights from EEG microstates," *Schizophr. Res.*, vol. 152, no. 2–3, pp. 513–520, 2014.
- [87] A. Stevens, W. Lutzenberger, D. M. Bartels, W. Strik, and K. Lindner, "Increased duration and altered topography of EEG microstates during cognitive tasks in chronic schizophrenia," *Psychiatry Res.*, vol. 66, no. 1, pp. 45–57, 1997.
- [88] M. Kikuchi et al., "EEG microstate analysis in Drug-Naive patients with panic disorder," *PLoS One*, vol. 6, no. 7, pp. 2–7, 2011.
- [89] T. Dierks, W. K. Strik, and K. Maurer, "Electrical brain activity in schizophrenia described by equivalent dipoles of FFT-data," *Schizophr. Res.*, 1995.
- [90] T. Koenig, C. Boesch, W. Strik, and T. Dierks, "Functional brain connectivity in combined EEG and fMRI," 14.Deutsches EEG / EP Mapping Meeting. 2005.
- [91] H. Yuan, V. Zotev, R. Phillips, W. C. Drevets, and J. Bodurka, "Spatiotemporal dynamics of the brain at rest - Exploring EEG microstates as electrophysiological signatures of BOLD resting state networks," *Neuroimage*, 2012.
- [92] T. Koenig et al., "Millisecond by millisecond, year by year: Normative EEG microstates and developmental stages," *Neuroimage*, vol. 16, no. 1, pp. 41–48, 2002.
- [93] R. D. Pascual-Marqui, C. M. Michel, and D. Lehmann, "Segmentation of Brain Electrical Activity into Microstates; Model Estimation and Validation," *IEEE Trans. Biomed. Eng.*, vol. 42, no. 7, pp. 658–665, 1995.

- [94] J. Wackermann, D. Lehmann, C. M. Michel, and W. K. Strik, "Adaptive segmentation of spontaneous EEG map series into spatially defined microstates," *Int. J. Psychophysiol.*, vol. 14, no. 3, pp. 269–283, 1993.
- [95] A. Kuhn, V. Brodbeck, E. Tagliazucchi, A. Morzelewski, F. von Wegner, and H. Laufs, "Narcoleptic Patients Show Fragmented EEG-Microstructure During Early NREM Sleep," *Brain Topogr.*, vol. 28, no. 4, pp. 619–635, 2015.
- [96] A. Khanna, A. Pascual-Leone, C. M. Michel, and F. Farzan, "Microstates in resting-state EEG: Current status and future directions," *Neuroscience and Biobehavioral Reviews*. 2015.
- [97] N. M. Drissi et al., "Altered Brain Microstate Dynamics in Adolescents with Narcolepsy," *Front. Hum. Neurosci.*, vol. 10, no. August, pp. 1–16, 2016.
- [98] R. H. Hashemi, W. G. Bradley, and C. J. Lisanti, *MRI: The basics*. 2012.
- [99] M. Neema et al., "T1- and T2-based MRI measures of diffuse gray matter and white matter damage in patients with multiple sclerosis," *J. Neuroimaging*, 2007.
- [100] J. B. M. Warntjes, O. Dahlqvist Leinhard, J. West, and P. Lundberg, "Rapid magnetic resonance quantification on the brain: Optimization for clinical usage," *Magn. Reson. Med.*, 2008.
- [101] D. Sulzer et al., "Neuromelanin detection by magnetic resonance imaging (MRI) and its promise as a biomarker for Parkinson's disease," *npj Park. Dis.*, vol. 4, no. 1, p. 11, 2018.
- [102] D. V. Clewett, T. H. Lee, S. Greening, A. Ponzio, E. Margalit, and M. Mather, "Neuromelanin marks the spot: identifying a locus coeruleus biomarker of cognitive reserve in healthy aging," *Neurobiol. Aging*, 2016.
- [103] L. Zecca et al., "Interaction of neuromelanin and iron in substantia nigra and other areas of human brain," *Neuroscience*, vol. 73, no. 2, pp. 407–15, Jul. 1996.
- [104] T. A. Bley, O. Wieben, C. J. Francois, J. H. Brittain, and S. B. Reeder, "Fat and water magnetic resonance imaging," *Journal of Magnetic Resonance Imaging*. 2010.
- [105] K. J. Friston, "Bayesian estimation of dynamical systems: An application to fMRI," *Neuroimage*, 2002.
- [106] C. Büchel, A. P. Holmes, G. Rees, and K. J. Friston, "Characterizing stimulus-response functions using nonlinear regressors in parametric fMRI experiments," *Neuroimage*, 1998.
- [107] S. Ulmer and O. Jansen, *fMRI: Basics and clinical applications*. 2013.
- [108] M. Havlicek, A. Roebroeck, K. Friston, A. Gardumi, D. Ivanov, and K. Uludag, "Physiologically informed dynamic causal modeling of fMRI data," *Neuroimage*, vol. 122, pp. 355–372, 2015.

- [109] J. S. Shimony, D. Zhang, J. M. Johnston, M. D. Fox, A. Roy, and E. C. Leuthardt, "Resting-state Spontaneous Fluctuations in Brain Activity. A New Paradigm for Presurgical Planning Using fMRI," *Acad. Radiol.*, 2009.
- [110] B. Krasnow et al., "Comparison of fMRI activation at 3 and 1.5 T during perceptual, cognitive, and affective processing," *Neuroimage*, 2003.
- [111] B. J. He and M. E. Raichle, "The fMRI signal, slow cortical potential and consciousness," *Trends Cogn. Sci.*, 2009.
- [112] M. N. Baliki, P. Y. Geha, A. V. Apkarian, and D. R. Chialvo, "Beyond Feeling: Chronic Pain Hurts the Brain, Disrupting the Default-Mode Network Dynamics," *J. Neurosci.*, 2008.
- [113] J. S. Damoiseaux et al., "Consistent resting-state networks across healthy subjects," *Proc. Natl. Acad. Sci.*, 2006.
- [114] D. Mantini, M. G. Perrucci, C. Del Gratta, G. L. Romani, and M. Corbetta, "Electrophysiological signatures of resting state networks in the human brain," *Proc. Natl. Acad. Sci.*, 2007.
- [115] S. B. Eickhoff et al., "Co-activation patterns distinguish cortical modules, their connectivity and functional differentiation," *Neuroimage*, 2011.
- [116] K. L. Ray et al., "ICA model order selection of task co-activation networks," *Front. Neurosci.*, 2013.
- [117] J. Britz, D. Van De Ville, and C. M. Michel, "BOLD correlates of EEG topography reveal rapid resting-state network dynamics," *Neuroimage*, vol. 52, no. 4, pp. 1162–1170, 2010.
- [118] F. Musso, J. Brinkmeyer, A. Mobascher, T. Warbrick, and G. Winterer, "Spontaneous brain activity and EEG microstates. A novel EEG/fMRI analysis approach to explore resting-state networks," *Neuroimage*, 2010.
- [119] V. D. Calhoun, J. Liu, and T. Adali, "A review of group ICA for fMRI data and ICA for joint inference of imaging, genetic, and ERP data.," *NeuroImage*. 2009.
- [120] N. Agarwal and P. F. Renshaw, "Proton MR spectroscopy - Detectable major neurotransmitters of the brain: Biology and possible clinical applications," *American Journal of Neuroradiology*. 2012.
- [121] S. R. Platt, "The role of glutamate in central nervous system health and disease - A review," *Veterinary Journal*. 2007.
- [122] S. T. Witt et al., "Evidence for cognitive resource imbalance in adolescents with narcolepsy," *Brain Imaging Behav.*, 2018.
- [123] G. Northoff et al., "GABA concentrations in the human anterior cingulate cortex predict negative BOLD responses in fMRI," *Nat. Neurosci.*, 2007.
- [124] S. A. Walter et al., "Positive allosteric modulator of GABA lowers BOLD responses in the cingulate cortex," *PLoS One*, 2016.

- [125] R. A. E. Edden, N. A. J. Puts, and P. B. Barker, "Macromolecule-suppressed GABA-edited magnetic resonance spectroscopy at 3T," *Magn. Reson. Med.*, 2012.
- [126] N. I. Keren et al., "Histologic validation of locus coeruleus MRI contrast in post-mortem tissue," *Neuroimage*, 2015.
- [127] K. Y. Liu, F. Marijatta, D. Hämmerer, J. Acosta-Cabronero, E. Düzel, and R. J. Howard, "Magnetic resonance imaging of the human locus coeruleus: A systematic review," *Neurosci. Biobehav. Rev.*, vol. 83, pp. 325–355, Dec. 2017.
- [128] L. Zecca, R. Pietra, C. Goj, C. Mecacci, D. Radice, and E. Sabbioni, "Iron and other metals in neuromelanin, substantia nigra, and putamen of human brain," *J. Neurochem.*, vol. 62, no. 3, pp. 1097–101, Mar. 1994.
- [129] R. Kumar, S. Delshad, P. M. Macey, M. A. Woo, and R. M. Harper, "Development of T2-relaxation values in regional brain sites during adolescence," *Magn. Reson. Imaging*, 2011.
- [130] S. Zhang, S. Hu, H. H. Chao, and C. S. R. Li, "Resting-State Functional Connectivity of the Locus Coeruleus in Humans: In Comparison with the Ventral Tegmental Area/Substantia Nigra Pars Compacta and the Effects of Age," *Cereb. Cortex*, 2016.
- [131] R. R. Markwald et al., "Impact of insufficient sleep on total daily energy expenditure, food intake, and weight gain," *Proc. Natl. Acad. Sci.*, 2013.
- [132] J. P. Chaput, "Is sleep deprivation a contributor to obesity in children?," *Eating and Weight Disorders*. 2016.
- [133] N. Dahmen, P. Tonn, L. Messroghli, D. Ghezel-ahmadi, and A. Engel, "Basal Metabolic Rate in Narcoleptic Patients," *Sleep*, pp. 2–4, 2009.
- [134] S. A. Porter, J. M. Massaro, U. Hoffmann, R. S. Vasan, C. J. O'Donnel, and C. S. Fox, "Abdominal subcutaneous adipose tissue: A protective fat depot?," *Diabetes Care*, 2009.
- [135] S. L. Hocking, D. J. Chisholm, and D. E. James, "Studies of regional adipose transplantation reveal a unique and beneficial interaction between subcutaneous adipose tissue and the intra-abdominal compartment," *Diabetologia*. 2008.
- [136] G. Torres-Villalobos et al., "Autologous subcutaneous adipose tissue transplants improve adipose tissue metabolism and reduce insulin resistance and fatty liver in diet-induced obesity rats," *Physiol. Rep.*, 2016.
- [137] V. A. Taylor et al., "Impact of meditation training on the default mode network during a restful state," *Soc. Cogn. Affect. Neurosci.*, 2013.
- [138] K. A. Garrison, T. A. Zeffiro, D. Scheinost, R. T. Constable, and J. A. Brewer, "Meditation leads to reduced default mode network activity beyond an active task," *Cogn. Affect. Behav. Neurosci.*, 2015.
- [139] G. Zhang, L. Yao, J. Shen, Y. Yang, and X. Zhao, "Reorganization of functional brain networks mediates the improvement of cognitive performance following real-time neurofeedback training of working memory," *Hum. Brain Mapp.*, 2015.

- [140] R. D. Pascual-Marqui, C. M. Michel, and D. Lehmann, "Segmentation of Brain Electrical Activity into Microstates; Model Estimation and Validation," *IEEE Trans. Biomed. Eng.*, 1995.
- [141] K. Nishida et al., "EEG microstates associated with salience and frontoparietal networks in frontotemporal dementia, schizophrenia and Alzheimer's disease," *Clin. Neurophysiol.*, vol. 124, no. 6, pp. 1106–1114, 2013.

Papers

The papers associated with this thesis have been removed for copyright reasons. For more details about these see:

<http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-153629>

FACULTY OF MEDICINE AND HEALTH SCIENCES

Linköping University Medical Dissertations, No. 1651, 2018
Department of Medical and Health Sciences (IMH)

Linköping University
SE-581 83 Linköping, Sweden

www.liu.se