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Investigating the links between sleep and complex diseases using polygenic risk and ultra-high field MRI

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List of abbreviations

1KGP	1000 Genomes Project
5-HT	serotonin
ACh	acetylcholine
AD	Alzheimer's disease
ANTs	Advanced Normalization Tools
APOE	Apolipoprotein E
Aβ	beta-amyloid
BAI	Beck Anxiety Inventory
BDI	Beck Depression Inventory
BDNF	Brain-Derived Neurotrophic Factor
BF	basal forebrain
BMI	body mass index
CSF	cerebrospinal fluid
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
EEG	electroencephalography
EMG	electromyogram
EOG	electrooculogram
ESS	Epworth sleepiness scale
fMRI	functional magnetic resonance imaging
FSL	FMRIB Software Library
FTG	gigantocellular tegmental field
GABA	Gamma-Aminobutyric Acid
GLM	general linear model
GLMM	generalized linear mixed models
GWAS	genome-wide association studies
HRC	Haplotype Reference Consortium
HRF	hemodynamic response function
IBD	identity by descent
ID	insomnia disorder
LC	locus coeruleus
LD	linkage disequilibrium
LDT	laterodorsal tegmentum

LH	lateral hypothalamus
MAF	minor allele frequency
MCH	melanin-concentrating hormone
MNI	Montreal Neurological Institute
MP2RAGE	Magnetization-Prepared with 2 RApid Gradient Echoes
MT-TFL	Magnetization Transfer-weighted Turbo-FLash
NE	Norepinephrine
NFTs	neurofibrillary tangles
NREM	non-rapid eye movement
NREMS	non-REM sleep
PDF	Probability Density Function
PPT	pedunculopontine tegmental nucleus
PRS	polygenic risk score
PSP	progressive supranuclear palsy
PSQI	Pittsburgh Sleep Quality Index
QC	Quality control
RBD	REM sleep behavioral disorder
REM	rapid eye movement
REMS	REM sleep
SCN	suprachiasmatic nucleus
SEff	sleep efficiency
SNP	single nucleotide polymorphisms
SOL	sleep onset latency
SPM	Statistical Parametric Mapping
SVD	singular value decomposition
SWA	slow wave activity
SWE	slow wave energy
SWS	slow wave sleep
TMN	tuberomammillary nucleus
TST	total sleep time
VLPO	ventrolateral preoptic nucleus
VTA	ventral tegmental area
WASO	wake after sleep onset

Abstract

Sleep-wake alterations may lead to sleep disorders, e.g. insomnia disorder (ID), and contribute to neurodegenerative diseases, including Alzheimer's disease (AD). These alterations may be present months, years or even decades before the onset of the disease. A putative mechanism of the bidirectional relationship between sleep-wake regulation, ID and AD involves the locus coeruleus (LC), a small brainstem nucleus and the main source of norepinephrine (NE) in the brain. In the doctoral project, we investigated early associations between sleep, ID and AD and assessed the contribution of the LC to the variability in sleep quality, with potential implications for these diseases. The first two studies combine genetics and electrophysiology to reveal associations between AD / ID risk and sleep phenotypes in youth. We conducted a quantitative analysis of nocturnal sleep electroencephalogram (EEG) in a relatively large cohort (N > 350) of healthy individuals aged < 35y and devoid of cognitive and sleep complaints and computed polygenic risk scores (PRS) for AD / ID for each individual. PRSs were obtained after genotyping, based on whole-genome common single nucleotide polymorphism assessments. The analyses showed that PRS for AD and PRS for ID were associated with sleep measures obtained from EEG and questionnaires. PRS for AD was positively associated with slow-wave energy (cumulated power in the 0.5–4 Hz EEG band) and daytime sleepiness. In contrast, PRS for ID was negatively associated with cumulated EEG power in the delta (0.5-4Hz) and theta (4-8Hz) bands across rapid eye movement (REM) and non-REM sleep and positively associated with the likelihood of falling asleep during the day. These results suggest that sleep variability may contribute to ID and AD many months, years or even decades before ID / AD symptoms onset. In addition, quantifying sleep alterations may be useful in assessing the risk of developing ID and AD. Sleep-wake alterations may, at least partly, involve the LC, thus in the third study, we investigated the role of the LC in sleep. In a multimodal study that included functional magnetic resonance imaging, EEG and questionnaires, we show that the activity of the LC, as probed in an attentional task during wakefulness, is correlated with subjective sleep quality as well as with the intensity of paradoxical sleep (theta band EEG spectral power during REM sleep) in healthy older (50-70y), but not in healthy younger individuals (18-30y). These findings support that the activity of the LC is reflected in the perception of the quality of sleep and in an essential oscillatory mode of REM sleep. Altogether, the three studies contribute to understanding the links between sleep, complex diseases and the LC.

Résumé

Les altérations du cycle veille-sommeil peuvent entraîner des troubles du sommeil, tel que l'insomnie (IN), et contribuent aux maladies neurodégénératives, y compris la maladie d'Alzheimer (MA). Ces altérations peuvent être présentes des mois, des années voire des décennies avant le début de la maladie. Un mécanisme putatif expliquant la relation bidirectionnelle entre la régulation veille-sommeil, IN et AD implique le locus coeruleus (LC), un petit noyau du tronc cérébral qui constitue la principale source de noradrénaline (NE) du cerveau. Dans ce projet de doctorat, nous avons étudié les associations précoces entre le sommeil, l'IN et la MA et évalué la contribution du LC dans la variabilité de la qualité du sommeil, ainsi que les implications cliniques potentielles de cette contribution. Les deux premières études combinent la génétique et l'électrophysiologie pour révéler des associations entre le risque de développer l'AD/DI et des phénotypes de sommeil chez des individus jeunes. Nous avons effectué une analyse quantitative du sommeil nocturne en utilisant l'électroencéphalogramme (EEG) au sein une cohorte relativement importante (N > 350) d'individus en bonne santé âgés de < 35 ans, sans troubles cognitifs et du sommeil, et pour lesquels nous avons calculé un scores polygéniques individuel de risque (PRS) pour l'AD/IN. Les PRS ont été obtenus après génotypage, sur la base d'évaluations de polymorphismes communs sur l'ensemble du génome. Les analyses ont montré que le PRS pour la MA et le PRS pour la IN étaient associées aux mesures du sommeil obtenues à partir de l'EEG et de questionnaires. Le PRS pour la MA était positivement associée à l'énergie des ondes lentes (puissance cumulée dans la bande EEG de 0,5 à 4 Hz) et à la somnolence diurne. En revanche, le PRS pour IN était négativement associé à la puissance EEG cumulée dans les bandes delta (0,5-4Hz) et thêta (4-8Hz) du sommeil paradoxal et du sommeil lent et positivement associé à la probabilité de s'endormir durant la journée. Ces résultats suggèrent que la variabilité du sommeil peut contribuer à l'IN et à la MA plusieurs mois, années, voire décennies avant l'apparition de symptômes. De plus, la quantification des altérations du sommeil pourrait s'avérer utile pour évaluer le risque de développer une IN ou une MA. Les altérations veille-sommeil peuvent, au moins en partie, impliquer le LC, donc dans la troisième étude, nous avons étudié le lien entre LC et sommeil. Dans une étude multimodale comprenant l'imagerie par résonance magnétique fonctionnelle, l'EEG et des questionnaires, nous montrons que l'activité du LC, telle que sondée dans une tâche attentionnelle à l'éveil, est corrélée à la qualité subjective du sommeil ainsi qu'à l'intensité du sommeil paradoxal (puissance spectrale thêta de la bande

EEG) chez les personnes âgées en bonne santé (50-70 ans), mais pas chez les personnes plus jeunes (18-30 ans). Ces résultats montrent que l'activité du LC se reflète dans la perception de la qualité du sommeil et dans un mode oscillatoire essentiel du sommeil paradoxal. Globalement, les trois études contribuent à la compréhension des liens entre sommeil, maladies complexes et LC.

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Theoretical introduction

Chapter 1: Sleep – general aspects

Sleep, which occupies about one-third of our life, can be seen as an essential state of body and mind, as a restorative brain function or even as an interesting or sometimes exhausting psychological experience. Given that it first appears relatively early in evolution and has become progressively complicated in tandem with the evolution of more complex neural systems, sleep appears to play a critical function in sustaining life. Sleep is characterized by diminished / absence of consciousness consists of repeating physiological patterns (stages), and it normally occurs at a particular time of a 24-hour cycle. It is reversible and self-regulating: the one who was sleep-deprived will tend to have a 'recovery sleep'. We become less aware of the environment when we sleep, but to a certain extent, and the degree of this loss of awareness depends on the sleep stage, the time spent in sleep, the duration and intensity of wakefulness before sleep.

This theoretical introduction aims to introduce several basic aspects of sleep-wake regulation which are important for the experimental part of this work. In Chapter 1 we start by illustrating the general organization of sleep as measured by polysomnography (PSG) and sleep scoring rules. Next, we outline the neurobiological and genetic foundations of sleep-wake control. We then discuss sleep in aging and disease. The second chapter of the introduction consists mainly of the published review and highlights the potential integrative role of the locus coeruleus in Alzheimer's disease (AD) and insomnia disease (ID).

Sleep stages and scoring

Stages of sleep are rhythmically recurring and defined by specific brain oscillations measured by electroencephalogram (EEG) together with electrooculogram (EOG) which records eye movements, and the electromyogram (EMG), a measure of muscle activity, which is placed on the face. In PSG, the EMG electrodes can be also placed on the legs to record the jerking leg movements for diagnosis of periodic limb movement disorder. Electrocardiogram (ECG) is recorded using two electrodes placed below the right clavicle and on the left side of the torso. The standard PSG also includes a snoring sensor, an oximeter, thoracic and abdominal effort belts.

The oscillations of the EEG are divided into wavebands according to their frequency: delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), sigma (12-16 Hz), beta (16-25 Hz), and gamma (25-50 Hz). The waveforms can be also described by their shape, amplitude and location on the head. Sleep is composed of several cycles including

two very distinct states: rapid-eye-movement (REM) and non-REM (NREM) states; NREM sleep is further divided into three stages that appear in a non-random order. REM sleep is also called paradoxical sleep, as its oscillatory pattern resembles the one of wakefulness. Contrary to wakefulness, it is characterized by sleep atonia (that is seen in EMG signal which becomes flat), irregularity of respiratory and heart rate and loss of temperature control, that can also be measured with PSG. This is the sleep stage when most people dream in the canonical understanding of dreams (Hobson, 2009). Mental activity also occurs during slow-wave sleep (SWS), but vivid dreams are less frequent than during REM sleep (Conduit et al., 1997).

In the past, several guidelines have been proposed to score sleep, and at the present time sleep is scored according to the American Academy of Sleep Medicine standard (Berry RB, Brooks R, Gamaldo CE, 2017) in 30-s epochs, by attributing a particular stage to each epoch: wake, stage 1, 2, 3 (N1, N2, N3 of NREM sleep) or REM sleep. Scoring implies several criteria of the frequencies and of the so-called grapho-elements, or waveforms, which are described hereafter. The visual representation of the EEG patterns observed in each stage is shown in **Figure 1.1**.

Wake

When the eyes are open, there are mainly low voltage fast frequencies present: beta and gamma, and it is called desynchronized or activated EEG. Wakefulness with eyes closed is usually characterized by the dominance of alpha rhythm at the occipital derivation. The EOG and EMG recordings also show high activity during wakefulness.

NREM

In N1, alpha activity drops to less than 50 percent of the 30-s epoch, and the rest of the signal consists of low voltage, mixed frequencies. If alpha activity is not well defined during wakefulness, then the signature of N1 may be sharp transient waves at the vertex, the top of the head. Slow rolling eye movements may be present in N1. Psychologically the transition into stage 1 is gradual and being asleep is normally recognized by most people after several minutes of EEG-defined sleep. Objectively sleep onset is often defined as 3 consecutive epochs of N1 or as the first epoch of N2. N2 is also composed of mixed frequency and low voltage oscillations, however, there are waveforms that distinguish N2 from N1: sleep spindles which are bursts of oscillatory activity at a frequency of 12-14 Hz of duration at least 0.5 sec, and K complexes which are slower high-voltage negative waves followed by positive waves.

In addition, the eye-movements are no longer present in N2. N2 is usually followed by N3, and the main signature of N3, the slow waves, may already be present in N2, but not more than in 20% of an epoch. N3 is characterized by higher amplitude (not less than 75 uV), slow delta waves, which comprise more than 20 percent of the epoch. This stage together with N2 is also known as slow-wave sleep (SWS). Noteworthy, the dominant frequency (the frequency that carries the maximum power among all frequencies found in the spectrum) of N2 is sigma, and the dominant frequency of N3 is delta.

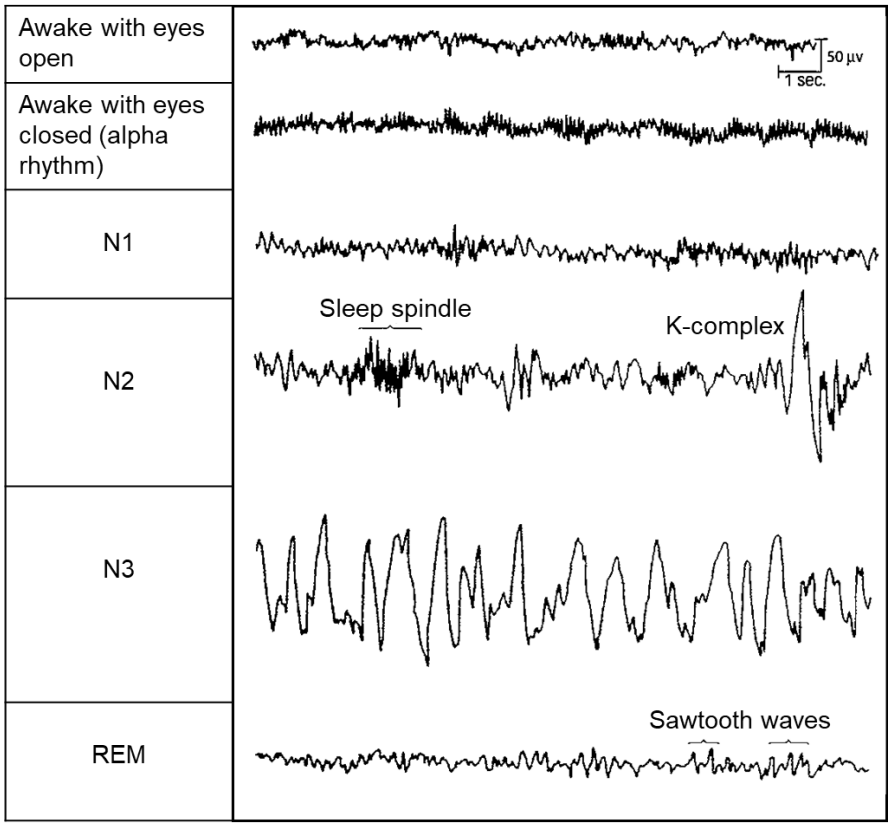

Figure 1.1. EEG oscillation patterns in sleep stages. EEG recordings in humans capture differences between vigilance states (wakefulness, NREM sleep, and REM sleep). Wakefulness is characterized by low-amplitude/high-frequency activity. NREM sleep begins in N1. In N2 and N3, both sleep spindles (7–14 Hz) and K-complexes are seen, as the EEG amplitude increases and frequency further slows. In N3, also known as slow wave sleep, strong delta (0.5–4 Hz) activity is evident, accompanied by a large increase in amplitude. During REM sleep, the EEG returns to a profile similar to wakefulness, with low-amplitude and high-frequency activity. Adapted from (Hitziger, 2015).

REM

Finally, REM sleep oscillations are composed of mixed frequency waves similar to N1 and wakefulness. The EEG may show “sawtooth waves”, of theta frequency, the dominant frequency of REM sleep. The widely-known signature of REM sleep is sharply peaked conjugate rapid eye movements seen at electrooculogram channels.

Sleep cycles

Night sleep normally comprises 4-6 NREM-REM cycles, with each cycle duration approximately 70-100 minutes and stage episodes often in the following order: N1, N2, N3, N2, REM. This idealized scenario varies, however. One may directly go to N2 stage, there may be no periods of N2 between N3 and REM, or one may not go to N3 before reaching REM. The cycles also differ across the night: the first episode of N3 depends on the time prior to wakefulness and is more intense (e.g. longer and contains more slow waves) at the beginning of the night, and then progressively dissipates (becomes shorter and shallower) in the next episodes (Halasz and Bodizs, 2013). In other words, the homeostatic sleep pressure that results from sleep need that accumulates during wakefulness (see the interaction between homeostatic and circadian factors in sleep regulation later), is the factor that determines the amount of slow waves generated during N3. The amount of REM sleep, in contrast to SWS, is greater in the second part of the night than at the beginning of the night, and depends much less on prior wakefulness. REM sleep is strongly affected by the circadian regulation of physiology across the 24h day and is closely coupled to the body temperature rhythm (Czeisler et al., 1980). (**Figure 1.2**).

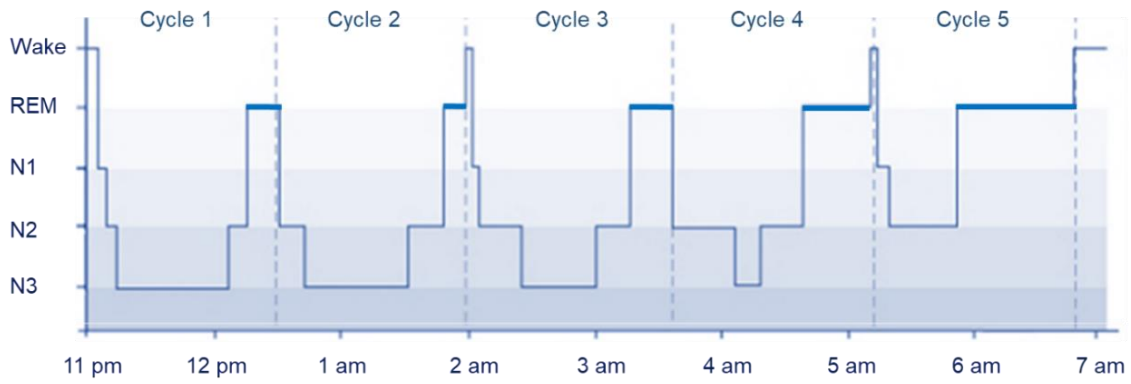

Figure 1.2. A sleep hypnogram of a healthy young adult. The graph represents sleep stages as a function of time during the night.

Physiology of REM and NREM sleep

The electrophysiological recordings of sleep capture essential differences in brain and body activity during wakefulness, REM and NREM sleep and reflect the uniqueness of physiological processes in each of them. Indeed, contrary to REM sleep, where the metabolic activity in the brain together with the cerebral blood flow is mostly preserved compared to wakefulness (except for frontal and parietal multimodal associative areas), it decreases progressively from N1 to N3. The functioning of the autonomic nervous system is however preserved in NREM sleep, with the dominance of the parasympathetic activity. Furthermore, one of the most prominent changes in the physiology of REM sleep is body muscle relaxation which originates from the peri-locus coeruleus area located in the brainstem which inhibits neurons responsible for muscle control in the spinal cord through the descending pathway. Blood pressure and heart rate show increased variability, respiration becomes irregular, and body temperature control is impaired: independent of whether the surrounding temperature is high or low, shivering or transpiration does not occur (Nicolau et al., 2000; Parmeggiani, 1992). In the brain, during REM sleep, some areas are even more active compared to wakefulness, and these areas include limbic and paralimbic circuits which are associated with emotions, motivation and memory. The eye movement periods are associated with brain areas responsible for emotions, arousal and attention.

The prominent differences in the physiology of the wakefulness, REM and NREM sleep, in their functions (Nicolau et al., 2000) as well as in the underlying neurobiology (Peever and Fuller, 2017) led some authors to describe humans as having three distinct states of consciousness: waking, REM sleep, NREM sleep (Hobson, 2009).

Neurobiology of sleep and wakefulness

The neurobiology of sleep and wakefulness is complex and organized at the genetic, molecular and cellular levels. Through a variety of neurotransmitters, which are known to either promote wakefulness or sleep, the signals are sent and received among various brain regions and the periphery. There have been several ideas put up to explain the change and continuity between wakefulness, NREM sleep, and REM sleep. All of these models combine the interaction of neurotransmitters into the complicated reciprocal connections among the major sleep-related regions. Additionally, a central pacemaker that is situated in the suprachiasmatic nucleus of

the hypothalamus and has the ability to communicate with peripheral tissues and cortical regions to promote the sleep–wake rhythm integrates a variety of environmental stimuli (Falup-Pecurariu et al., 2021).

Sleep cyclicity. Circadian and homeostatic components of sleep

One of the definitions of sleep emphasizing its cyclicity is the one of Henry Piéron more than a century ago: 'It is a physiological periodically necessary state, with cyclicity relatively independent from the external conditions and characterized by the interruptions of the complex sensory and motor relations that link the individual with his environment' (Piéron, 1913). Today we know that sleep mainly depends on the interaction between two main factors: the circadian timing system and homeostatic sleep pressure (Borbély, 1982).

Circadian regulation of sleep

The fact that sleep is typically restricted to a specific time of day or night in most species is a very important feature of sleep. The need to rest and the need to respond adequately to alternating periods of light and dark are significant factors that influenced how species evolved (Keene and Duboue, 2018). Having a circadian clock mechanism that controls behavior and physiological processes is one way to correspond to these needs. A brief description of the internal clock is presented below.

The word 'circadian' comes from the Latin 'circa-diem', which means 'approximately 1 day'. The circadian system is modulated by an internal master clock, the suprachiasmatic nucleus (SCN) (Hastings et al., 2018), which is located in the anterior part of the hypothalamus. Most tissues of the body have rhythmic properties, and their overall regulation is provided by the SCN. In its turn, the SCN rhythm results from the cycles in the expression of the clock genes. The SCN is located just above the optic chiasm in which the pathways from the retina converge. The activity of the SCN does not only rely on its inherent rhythm, but is partially regulated by external cues, the most robust one being the light-dark cycle (day-night alternation). The retina contains a particular subset of retinal ganglion cells (Schmidt and Kofuji, 2008), which collect light signal of rods and cons, but are also intrinsically photosensitive because they express a blue-sensitive photopigment called melanopsin. The combination of light information arising from these different photoreceptors is transmitted to the SCN through the retino-hypothalamic tract. The

master clock sends in turns messages to other ‘clocks’ that are located in different parts of the body, and influences sleep and behavior (Welsh et al., 2010).

The SCN innervates the pineal gland (a small gland located near the center of the brain, where the two halves of the thalamus join) through an indirect pathway. The cells of the SCN are responsible for the synthesis and secretion of melatonin. The synthesis of melatonin at night is activated by norepinephrine (NE) released from postganglionic sympathetic nerve endings that terminate in the vicinity of the pinealocyte processes (Ramachandran, 2002). Melatonin is released from the pineal gland and circulates in the bloodstream, in effect providing this information (that it is dark and night-time) to the rest of the body (Benloucif et al., 2016). It also provides a kind of negative feedback to the SCN itself, which is very sensitive to melatonin: one type of melatonin receptors in the SCN decreases the arousing properties of the SCN. In the early morning, the dopamine receptors on the outside of the pineal gland work alongside norepinephrine receptors to stop – almost completely – the melatonin-secretion signal (González et al., 2012).

Homeostatic regulation of sleep

Homeostatic sleep pressure builds up in our body as our time awake increases and tracks the previous sleep-wake history. It is believed that adenosine is one of the most important ‘sleep factors’ together with many other neurotransmitters and neuropeptides involved in sleep homeostasis. Manipulating the adenosine system leads to changes in sleep (reviewed in (Huang et al., 2011)). Adenosine is assumed to be generated inside cells or on their surface, primarily through the breakdown of adenine nucleotides, and is neither stored nor released as a traditional neurotransmitter. In the cortex and basal forebrain, the extracellular concentration of adenosine rises during extended awake and falls during the sleep recovery phase. Adenosine is therefore suggested to function as a homeostatic sleep regulator and as a bridge between the humoral and neuronal systems of regulating sleep and wakefulness (Huang et al., 2011).

Besides molecular factors, the structure of synapses is also considered to be involved in sleep homeostasis, through the synaptic homeostasis hypothesis (Tononi and Cirelli, 2006) which links the homeostatic regulation of sleep to mechanisms of neuronal plasticity and learning. According to it, one of the fundamental functions of sleep is the restoration of synaptic function which is challenged by synaptic strengthening triggered by learning during wake and by synaptogenesis during development. During wake neurons fire and increase the strength and size of their

synapses, particularly in response to salient events. This increase in synaptic strength comes with definite costs at the cellular and systems levels: it means higher energy consumption, but also reduces the selectivity of neural responses with a potential saturation of the ability to learn. During sleep, when we are essentially disconnected from the environment, the opportunity arises to downscale the synaptic strength and surface which restores neuronal selectivity and enhances signal-to-noise ratios promoting memory consolidation. Presumably, neurons that fire particularly stronger during sleep are preferentially protected from synaptic depression mechanisms.

According to this theory, the synaptic downscaling occurs during SWS rather than REM sleep, and the slow oscillations which have higher amplitude at the beginning of the night, reflect the overall increase in the synaptic strength and represent a mechanism for its downscaling. This hypothesis remains partially true in our days and its postulates have been questioned in subsequent studies by suggesting that the synaptic downscaling is accompanied by local upscaling across the NREM-REM cycle, and that the processes during REM sleep should be equally taken into consideration as downscaling in hippocampal networks might be mediated through REM sleep theta rather than SWS (Chauvette et al., 2012; Grosmark et al., 2012). Later it was shown that REM sleep regulates synapse function, facilitating memory and learning: REM sleep appears to selectively prune and maintain new synapses linked to specific types of motor learning for instance (Li et al., 2017). The theory has also been criticized for the lack of functional significance and the insufficient understanding of its mechanisms (Frank, 2012).

Homeostatic-circadian interaction

In the model proposed by Borbély (Borbély, 1982), the circadian component (Process C) is represented by a sinusoidal oscillation that reaches its peak close to our habitual sleep time, and the homeostatic component (Process S) shows an exponential increase during wakefulness and exponential decrease during sleep. The most prominent marker for the decrease of homeostatic sleep drive (descending limb of S) is the dissipation of the slow wave activity (spectral power in the 0.5–4 Hz range, SWA) in NREM sleep. In waking, theta activity is a marker of the rising limb of S (reviewed by (Borbély and Achermann, 1999)). The homeostatic and circadian factors interact, modulating our physiology, behavior and cognition. When S approaches the range's lower boundary it triggers awakening, near the upper boundary it triggers sleep. The circadian component counteracts the increase in S up

to the so-called evening wake-maintenance zone in order to ensure a consolidated ~16h wakefulness episode; then the ascending segment the circadian turns into a sleep-promotinging signal to ensure continuous ~ 8h sleep episode even when sleep pressure is largely dissipated at the end of the night (**Figure 1.3**). The interplay between these 2 processes not only determines alertness daily fluctuations, but affects higher cognitive functions, in such way that when wakefulness is extended into the biological night, the circadian system no longer opposes the high need for sleep, and so cognitive performance is compromised (Dijk and Archer, 2010).

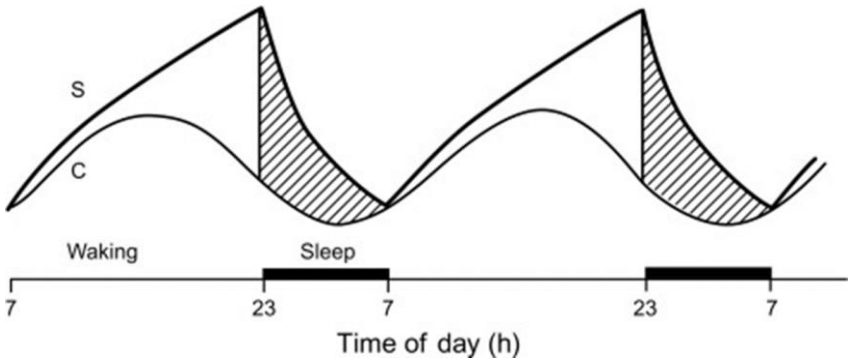

Figure 1.3. Schematic representation of the Two-Process Model of Sleep Regulation. Adapted from (Borbély, 1982).

Neural circuits of sleep-wake regulation

Sleep is a reversible physiological state which involves a specific pattern of cerebral activity and goes far beyond just a down-scaled version of one of wakefulness. In the last few years, it was proposed that the sleep and wake circuitries primarily use fast neurotransmitters such as glutamate and GABA, and slow neurotransmitters including monoamines, acetylcholine, peptides, have a modulatory role (Saper and Fuller, 2017). The actual theory is based on results of animal model studies using lesions and opto- and chemogenetic excitation and inhibition and is a reconsidered version of the previously favored hypothesis postulating that the wake-promoting influence mainly arises from monoaminergic and cholinergic neurons in the upper brainstem which innervates the thalamus and hypothalamus, basal forebrain and then projects to the cerebral cortex (Saper et al., 2005).

Wakefulness and sleep

The pathways promoting wakefulness and sleep are depicted in the left panel of **Figure 1.4**, and the structures which are primarily responsible for generation of wakefulness are shown in red: the glutamatergic and cholinergic neurons in the

parabrachial nucleus and pedunculopontine tegmental nucleus (PPT) and in supramammillary nucleus project to the basal forebrain (BF); the BF also includes cholinergic and GABAergic neurons that innervate the cerebral cortex (Kroeger et al., 2017; Pedersen et al., 2017). Interestingly, the parvalbumin of GABAergic neurons in the BF was shown to strongly promote wake and the associated faster EEG rhythms (Anaclet et al., 2015) while stimulation of the cholinergic neurons reduced slow waves in EEG (Chen et al., 2016). In addition, GABAergic neurons in the lateral hypothalamus (LH), which are shown in purple on the figure, have recently been proposed to promote wakefulness (Venner et al., 2016). Other neurotransmitters, such as norepinephrine in the locus coeruleus (LC), serotonin in the dorsal raphe nucleus, acetylcholine in PPT, histamine in tuberomammillary nucleus (TMN), and orexin in the LH, modulate the action of the putative flip-flop mechanism of switching between wakefulness and NREM sleep.

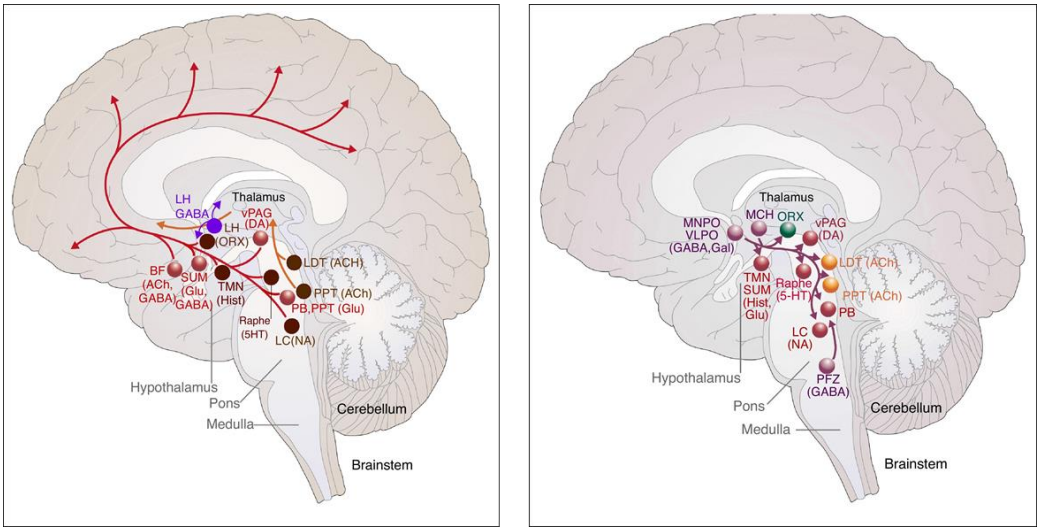

Figure 1.4. Wake and sleep promoting pathways. Left: core wake promoting nuclei are shown in red while nuclei that play modulatory role are shown in brown and puple. Right: Sleep promoting nuclei are shown in purple while wake promoting nuclei are shown in red, yellow, and green. 5HT: serotonin, Ach: acetylcholine, Hist: histamine, DA: dopamin LDT: laterodorsal tegmental nucleus, NA: noradrenaline, ORX: orexin, TMN: tuberomammillary nucleus, LC: locus coeruleus VLPO: Ventrolateral preoptic nucleus, MnPO: median preoptic nucleus, PFZ: parafacial zone, MCH: melanin-concentrating hormone, PB: parabrachial nucleus, PPT: pedunculopontine tegmental nucleus, BF: basal forebrain, SUM: supramammillary nucleus, vPAG: ventral periaqueductal gray matter, LH: lateral hypothalamus. Adapted from (Saper and Fuller, 2017).

The pathways promoting sleep are depicted in the right panel of **Figure 1.4**. NREM sleep is mainly promoted by the ventrolateral preoptic nucleus (VLPO): the neurons in these nuclei mostly discharge during NREM sleep, but they are also essentially silent during wake (España and Scammell, 2011). These neurons release GABA and galanin, but it is not established whether it is due to GABA, galanin, or to release of some other transmitter (Kroeger et al., 2018). Another site is the parafacial zone GABAergic neurons in the medulla which induce sleep by inhibiting the parabrachial glutamatergic arousal neurons. Certain areas of the basal forebrain and lateral hypothalamus are additional brain regions that induce NREM sleep via GABAergic neuromodulation (España and Scammell, 2011).

Wakefulness-NREM switch

The switch between wakefulness and NREM is thought to be controlled by the interaction of the sleep-promoting and wake-promoting nuclei. Ventrolateral preoptic (VLPO) and median preoptic nuclei send inhibitory projections (by releasing GABA) to arousal-promoting nuclei. Wake-promoting nuclei the neurons of which release serotonin (dorsal raphe), norepinephrine (LC) and are involved in excitatory pathways in the cerebral cortex, in turn send inhibitory signals to the VLPO. The result of this mutual inhibition is alternation between sleep and wakefulness. In addition, the lateral hypothalamus that releases orexin / hypocretins, takes part in the stimulation of the arousal nuclei and is active during wakefulness (Gompf and Anaclet, 2020).

REM sleep and REM-NREM switch

Glutamatergic neurons in the subcoeruleus region, which corresponds to sublaterodorsal region in rodents (Boeve et al., 2007a) together with cholinergic neurons in the laterodorsal tegmental and pedunculopontine nuclei (REM-on neurons), seem to be responsible for REM sleep generation (Lu et al., 2006; Scammell et al., 2017) The neurons in the subcoeruleus region also activate the inhibitory interneurons in the medulla and the spinal cord inducing the muscle atonia during REM sleep. GABAergic neurons in the ventrolateral periaqueductal gray matter and lateral pontine tegmentum (REM-off neurons) fire during NREM sleep and inhibit the transition to REM sleep. REM-on are reciprocally connected with REM-off neurons and together they form the basis of a pontine REM ‘flip-flop’ switch. This flip-flop REM-NREM switch is modulated by other neurotransmitters: norepinephrine from the LC and serotonin from the dorsal raphe nucleus excite REM-off and inhibit REM-on neurons and thus indirectly inhibit REM sleep; ACh in the PPT and laterodorsal tegmentum (LDT) indirectly promote REM sleep in a similar way.

Furthermore, REM-off population is excited by orexin from the LH and is inhibited by GABA from VLPO which allows inhibition or promoting entry into REM sleep (Saper et al., 2010).

An extended model for this flip-flop sleep-wake transitions (Saper et al., 2010) was proposed in (Eban-Rothschild et al., 2017). They aimed to take into account sleep phenomena like local sleep (Huber et al., 2004; Krueger et al., 2019) and sleep inertia, as well as dissociated arousal states, like narcolepsy or REM behavioral disorder, which cannot be explained in terms of the binary states model. They suggested that the hypocretin neurons in the LH serve as an integrator of the information flow from multiple variables that include the functional connectivity between the neurotransmitter systems and physiological factors such as circadian phase, sleep history, emotional status, energy demand and others. The essential part of this model is the time scale of integration, i.e. how long does it take for an integration neuron to decide 'to sleep or not to sleep'. In simple terms, sleep-wake transitions are not purely consequences of neuro-biological mechanisms *per se*, but occur following integrative probabilities that account for physiological and psychological factors.

Genetics of sleep

Heritability of sleep and sleep disorders

Genetics can be used to address questions about sleep regulation, sleep function, abnormal sleep, and, importantly, sleep disorders. It is established that sleep and sleep disorders are at least partly genetically controlled: even if the environment can affect the quantity and quality of sleep, the heritability of sleep variables supports genetic regulation. Studies in twins have demonstrated genetically determined variance in sleep EEG patterns (Ambrosius et al., 2008). For example the heritability was estimated to reach 96% in specific frequency bands (8-16 Hz) (De Gennaro et al., 2008), which is independent of sleep need and intensity, potentially making it one of the most heritable traits in humans. At the same time, the SWS which is primarily under homeostatic control is also influenced by genetic factors, with estimates of heritability around 50% (Linkowski, 1999). Furthermore, several studies reported heritability of sleep duration, measured both by EEG and subjectively, as well as subjective sleep quality (Gedda and Brenci, 1983; Partinen et al., 1983). A systematic review established that about 46% of the variability in sleep duration and about 44% of the variability in sleep quality is genetically determined and varies with age: it becomes higher in adulthood compared to childhood, but the highest estimates are in

adolescence (Kocevska et al., 2021). Sleep onset latencies, awakening measures, stage changes and amounts of REM sleep, as well as temporal patterns of REM during REM sleep are also heritable to a certain extent (Webb and Campbell, 1983). Twin studies have also detected a genetic component for a range of sleep disorders including insomnia, restless leg syndrome, obstructive sleep apnea, and narcolepsy (Barclay and Gregory, 2013). A meta-analysis revealed the heritability of insomnia to be 40 % (Barclay et al., 2021).

Sleep genes

Despite an increasing number of studies and databases related to sleep, a coherent picture on sleep genetic architecture has yet to emerge. Single gene mutations which are responsible for sleep phenotypes as well as sleep disorders are rare (e.g., fatal familial insomnia) and commonly, sleep disorders involve both genetic, environmental factors, and complex interactions between them (Tafti et al., 2005). Below are some examples of genes that regulate EEG-measured sleep features.

A functional polymorphism of the adenosine deaminase (ADA) gene (coding for a protein involved in purine metabolism; its mutation leads to immunodeficiency), is associated with variability in sleep architecture and the sleep power spectrum. In people with the G/A genotype compared to those with the G/G genotype, slow-wave sleep lasts longer and is more intense. Specifically, those with the G/A genotype slept for longer periods of time in slow-wave sleep and had higher sleep delta powers than those with the G/G genotype (Rétey et al., 2005). The Val66Met functional polymorphism of brain derived neurotrophic factor (BDNF) is also implicated in EEG sleep measures variability. Met carriers (Val/Met and Met/Met genotypes) showed decreased spectral power in the alpha band in stage 1 and decreased theta power in stages 2 and 3 of NREM (Guindalini et al., 2014). Another example of sleep genes is a family of CLOCK genes generally implicated in circadian rhythms generation (Dunlap, 1999; Reppert and Weaver, 2001). It was shown that a variable-number tandem-repeat polymorphism in one of them, PERIOD3 (PER3), is related to sleep homeostasis: compared to individuals with the shorter allele (PER3 4/4, i.e. with 4 repeats), those who were homozygous for the longer repeat (PER3 5/5, i.e. with 5 repeats) showed shorter sleep latencies, longer total sleep times and higher slow wave activity in NREM sleep (Viola 2007).

A large variety of sleep phenotypes including time spent in different sleep states, their distribution over the 24-h cycle, and the frequency-specific characteristics of EEG, as well as the global nature of sleep being a circuit-driven behavior (contrary to circadian

rhythms that rely on the cell-autonomous processes and thus can be better tracked through genetic approach) makes it impossible to find core “sleep genes” which would determine sleep. Another confound is the pleiotropic effects of genes involved in sleep regulation and in sleep disorders: for example, variants of genes implicated in restless leg syndrome, are usually related to neural development; sleep disorders are genetically linked with psychiatric and mental disorders (Veatch et al., 2017). The more realistic approach therefore is to reveal sets of genes or gene pathways which modulate a specific aspect of sleep. Related to this, genome-wide association studies (GWAS) (Uffelmann et al., 2021) are extensively used for discovering genetic variants associated with a specific trait or disease. With increasing GWAS sample sizes, the number of associated variants grows steadily. GWAS results can be used in a variety of applications, and one of them is assessing an individual’s risk for a disease based on their DNA profile. A recent study showed that for complex diseases, the genomic risk prediction from polygenic risk score (PRS) is comparable to the one predicted from monogenic mutations (Khera et al., 2018), and the genomic risk prediction may soon be used for clinical purposes and personalized medical care. This tool is used in our published experimental paper for the prediction of insomnia risk in people without sleep complaints.

Sleep and aging; insomnia and AD

Sleep architecture over the lifespan

Sleep changes across the lifespan and a few generalizations can be made to visualize the development of sleep **(Figure 1.5)**. Total sleep time is greatest at around birth and slowly declines over childhood. It then levels off in adolescence and early adulthood, before declining again with aging. Also, the percentage of time spent in sleep stages varies. The percentage of REM sleep follows a similar trend to total sleep: it is quite high in childhood, declines until puberty, is quite constant during adulthood, and then declines as people become older, but with significant variability. Children have the highest percentage of slow-wave sleep, which subsequently gradually declines through adolescence, adulthood, and old life. In contrast, sleep continuity deteriorates with age: the number of awakenings increases steadily over the course of a lifetime and is relatively modest early in life.

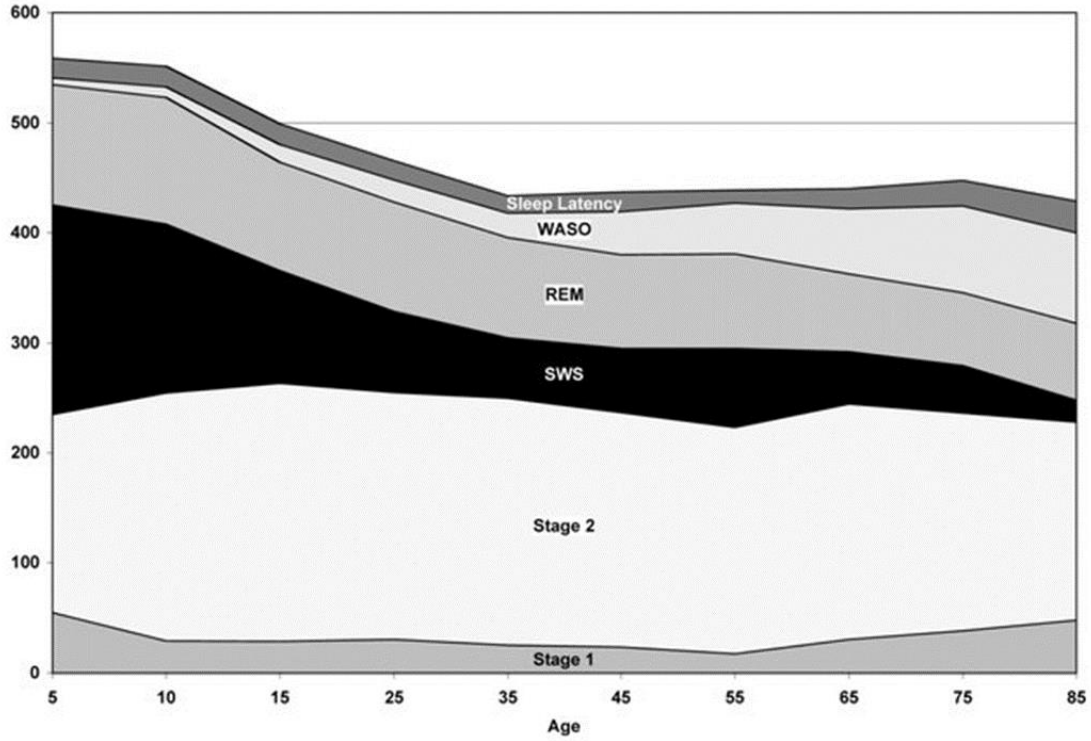

Figure 1.5. Changes in sleep architecture across the lifespan. The meta-analysis included 65 studies representing 3,577 subjects aged 5 years to 102 years. Adapted from (Ohayon et al., 2004).

Young vs older adults

At the older age, people tend to evaluate their sleep as being poorer, they have troubles in falling asleep and maintaining it. Electroencephalography (EEG) reveals alterations at the macro-level, characterized by shorter total sleep time (Klerman and Dijk, 2008), more fragmented (Van Cauter et al., 2000) and fragile sleep (Zepelin et al., 1984) with shorter cycles (Conte et al., 2014), and the micro-level, including reduced quantity and quality of sleep oscillations (Mander et al., 2017), Specifically, the SWA reductions are most prominent (Landolt et al., 1996; Landolt and Borbély, 2001; Mander et al., 2013), with both the amplitude and the density of slow waves decrease (Carrier et al., 2011). Besides, it has been suggested that, in the elderly, the homeostatic regulation is impaired (Dijk et al., 1999): the SWS rebound is blunted (Landolt and Borbély, 2001), the rate of decay of the homeostatic pressure declines (Landolt et al., 1996) and sleep fragmentation may be a consequence of this. Older people have more fragmented sleep-wake cycle that is observed both during the night and the day reflected in early awakenings and napping during the day (Foley et al., 2007). The naps occur spontaneously rather than planned and result from the excessive sleepiness. An increase in daytime sleepiness can be seen using the Multiple Sleep Latency Test (MSLT). This test is normally used for diagnostic

purposes in sleep clinics, but can also be used in research to measure sleep need. The test consists of four or five 20-minute nap opportunities set two hours apart. The sleep latencies are recorded for each trial. The increased daytime sleepiness in the elderly may mean that the shorter nocturnal sleep is not due to less sleep need but due to inability to consolidate sleep. At the same time, health status is a large confound in the increased sleepiness, and the question if the older people need less sleep or are just not able to generate sufficient sleep is still debated (Klerman and Dijk, 2008; Mander et al., 2017). Noteworthy, the balance between how much sleep we need and how much sleep we get must be related to the functions of sleep which are not fully understood and include memory consolidation and brain metabolites clearance. Thus, this balance would be partially best defined by the goodness of daytime functioning measured both objectively (cognitive tests) and subjectively (questionnaires). Sleep health, according to Daniel Buysse, the author of the well-known questionnaire for defining subjective sleep quality, the Pittsburgh Sleep Quality index (PSQI, (Buysse et al., 1989) is a multidimensional pattern of sleep-wakefulness, adapted to individual, social, and environmental demands, that promotes physical and mental well-being. Good sleep health is characterized by subjective satisfaction, appropriate timing, adequate duration, high efficiency, and sustained alertness during waking hours.

Sleep, insomnia, Alzheimer's disease

There is however a large inter-individual variability in the degree of age-related sleep disruption: some people preserve good sleep while others show dramatic alterations in the sleep-wake cycle (Redline et al., 2004; Vitiello, 2009). For example, it was observed the for some older adults the daytime rating of subjective sleepiness diminishes (Dijk et al., 2010). Besides, it is sometimes difficult to separate changes in sleep from the effects of illnesses or medications, and the sleep-wake disruption is more prominent in the presence of comorbid conditions such as depression and sleep disorders (Foley et al., 2007; Vitiello, 2009).

Insomnia disorder (ID) whose diagnosis is primarily based on subjectively reported sleep complaints, such as difficulties in falling asleep, maintaining sleep continuity, and early morning awakenings, is among the most prevalent sleep disorders. ID is significantly more prevalent in older age; it has been estimated that as many as 50% of older adults complain about difficulty initiating or maintaining sleep (Crowley, 2011). ID increases with age, is comorbid with psychiatric disorders and the mechanisms underlying altered sleep in ID are still a matter of debate. The

vulnerability to develop ID cannot be explained by homeostatic deficiency or by the circadian dysfunction alone (Van Someren, 2021). At complement, an increasing amount of reports convey to the fact that both subjectively and objectively reported deviations in sleep are underlined by hyperarousal in ID (Feige et al., 2008). Hyperarousal resembles the state of acute anxiety or emotional distress and is commonly mentioned as the key subjective complaint experienced by people with ID (Bonnet and Arand, 2010; Riemann et al., 2010). The hyperarousal theory assumes an interplay between genetic vulnerability for sleep-wake dysregulation, psychological stressors (traumatic life events or intense emotional experiences) and learned negative sleep misconceptions (Riemann et al., 2010), while an overall state of hyperarousal is considered as a major predisposing factor for ID. Accordingly, behavioral, neuroendocrine, electrophysiological and neuroimaging studies observed increased levels of arousal in ID both during night and daytime (Riemann et al., 2010).

ID and insomnia-like complaints increase the risk of cognitive decline (Virta et al., 2013) and are associated with all-cause dementia including AD (Bubu OM, Brannick M, Mortimer J, Umasabor-Bubu O, Sebastião YV, Wen Y, Schwartz S, Borenstein AR, Wu Y, Morgan D, 2017; de Almondes et al., 2016; Lim et al., 2013). A recent review illustrated that sleep deprivation and insomnia are linked to the pathogenesis of Alzheimer's disease (AD) and may have an impact on its symptoms and development (Sadeghmousavi et al., 2020). There is a bidirectional detrimental link between AD and sleep (Van Egroo et al., 2019).

A putative neural substrate at the intersection between sleep-wake regulation, ID and AD is the locus coeruleus (LC), a small brainstem nucleus, the main source of norepinephrine (NE) in the brain. In the next chapter we will focus on the LC: we will present and discuss findings that support the major role of the LC-NE system at different levels of sleep-wake organization, ranging from its involvement in the overall architecture of the sleep-wake cycle to its associations with sleep microstructure, while accounting for the intricate neuroanatomy surrounding the LC. We will discuss the emerging opportunities to investigate LC-NE mediated relationships between healthy sleep, ID and AD. The text is mainly taken from a published review that is placed in the **Appendix 1.**

Chapter 2: Locus coeruleus: its roles in sleep and diseases[1]

The LC-NE system: structure, functions, and proxy measures

The LC is a nucleus located bilaterally in the dorsal area of the rostral pons, lateral to the fourth ventricle, extending from the lower level of the inferior colliculus to the motor nucleus of the trigeminal nerve (Sharma et al., 2010). The name *locus coeruleus* (Latin for 'blue spot') originates from the blue color observed during the initial histological investigation of this brain structure, which is due to the presence of neuromelanin granules within LC NE-containing neurons. Despite its small number of neurons and its modest size (~14.5 mm long and ~2.5 mm thick in adult humans) (Beardmore et al., 2021), the LC constitutes the primary source of NE for the central nervous system (Poe et al., 2020). LC neurons possess immensely ramified axons that allow for extensive projections and release of NE over the whole brain, including the hippocampus, amygdala, thalamus, and neocortex (**Figure 2.1**), with the exception of a few dopaminergic basal nuclei (Schwarz et al., 2015). Mixed findings were reported with regards to age-related effects on LC-NE structural alteration in *post-mortem* investigations of non-pathological aging, whereas several *in vivo* studies suggest an inverted U-shape curve between MRI-assessed LC signal and increasing age, with the 5th decade as a tipping point (Beardmore et al., 2021; Liu et al., 2017). By contrast, LC-NE neurodegeneration is clearly evident in neurodegenerative conditions, such as AD, with patients consistently displaying LC neuronal loss compared to controls, as early as in the prodromal stages of the disease (Beardmore et al., 2021).

Three families of receptors (α_1-, α_2-, and β-adrenergic receptors), with either excitatory (α_1, β) or inhibitory (α_2) effects on cell signaling, have been identified as widespread binding sites for the action of NE (Hein, 2006). Given the neuromodulatory properties of NE, the primary role of the LC-NE system is to modulate its targets in order to induce and/or maintain behavioral states and state-dependent cognitive processes (Poe et al., 2020). The LC-NE system is therefore involved in regulating a broad range of brain functions and processes (Chandler et

[1] The text of this chapter comes mainly from a review I published as co-first author in 2022 which was entitled "Importance of the locus coeruleus-norepinephrine system in sleep-wake regulation: Implications for aging and Alzheimer's disease", and published in the journal Sleep Medicine Reviews. The review included an introduction section that was removed in the present manuscript because it repeated in part the aspects covered in Chapter 1. The review was further not making links with insomnia, so I added section on LC and insomnia to the text. The original review can be found in the Appendix 1 of the present thesis.

al., 2019), including arousal, attention, autonomic activity, emotional regulation, memory, sensory processing, nociception, or stress.

The LC-NE system fulfills its functions through two modes of functioning, defined by either tonic or phasic discharge patterns (Devilbiss, 2019). During wakefulness, tonic LC-NE activity is state-dependent and covaries with arousal levels (Aston-Jones and Bloom, 1981; Aston-Jones et al., 2007). In addition to tonic discharge rates, phasic bursts of LC-NE neurons are elicited when confronted with novel or salient stimuli, and this phasic LC activity is mirrored by frequency increases in electroencephalography (EEG) and behavioral markers of attention and alertness (Holland et al., 2021). Beside electrophysiological recordings, the activity of the LC-NE system can be indirectly assessed through proxy measures derived from LC-NE neuronal activity, such as extracellular levels of NE (Mather, 2021) or variations in pupil size (Joshi and Gold, 2020).

The LC-NE system and the anatomy of the sleep-wake circuitry

The LC-NE system is part of the reticular formation, a network of nuclei composing the ascending and descending pathways. While the latter deals with regulation of sensory and motor aspects (*e.g.* nociception, muscular tonus) and will not be considered here, the position of the LC-NE system in the former is the focus of the present section. In addition to the LC, the ascending arousal system includes the pedunculopontine (PPT) and laterodorsal tegmental (LDT) nuclei, the raphe nucleus, and the ventral tegmental area (VTA), releasing acetylcholine (Ach), serotonin (5-HT) and dopamine, respectively. Together with the basal forebrain (Ach), the hypothalamus (histamine and orexin), and the action of fast neurotransmitters (glutamate and GABA), these wakefulness-promoting systems are opposed to the sleep-promoting action of GABAergic and galaninergic neurons of the preoptic area, melanin-concentrating hormone (MCH) neurons of the hypothalamus, and GABAergic neurons of the parafacial zone. Altogether, this intricate sleep-wake circuitry constitutes the neurobiological underpinning of sleep and wakefulness regulation (for a detailed visualization of the sleep-wake circuitry and pathways, see (Saper and Fuller, 2017)).

More than 40 years ago, retrograde tracing studies suggested that LC-NE neurons were topographically organized according to their target projection sites (Mason and Fibiger, 1979; Poe et al., 2020), and this modular architecture was later confirmed with the use of viral-genetic approaches (Schwarz et al., 2015). Within the sleep-wake neurobiological network, LC-NE neurons were found to project to cholinergic

and GABAergic neurons of the basal forebrain, GABAergic neurons of the ventrolateral preoptic area (VLPO) in the anterior hypothalamus, orexinergic neurons of the lateral hypothalamus, serotoninergic neurons of the dorsal raphe, and cholinergic neurons of the PPT nucleus (Lew et al., 2021; Samuels and Szabadi, 2008; Saper and Fuller, 2017) (**Figure 2.1**).

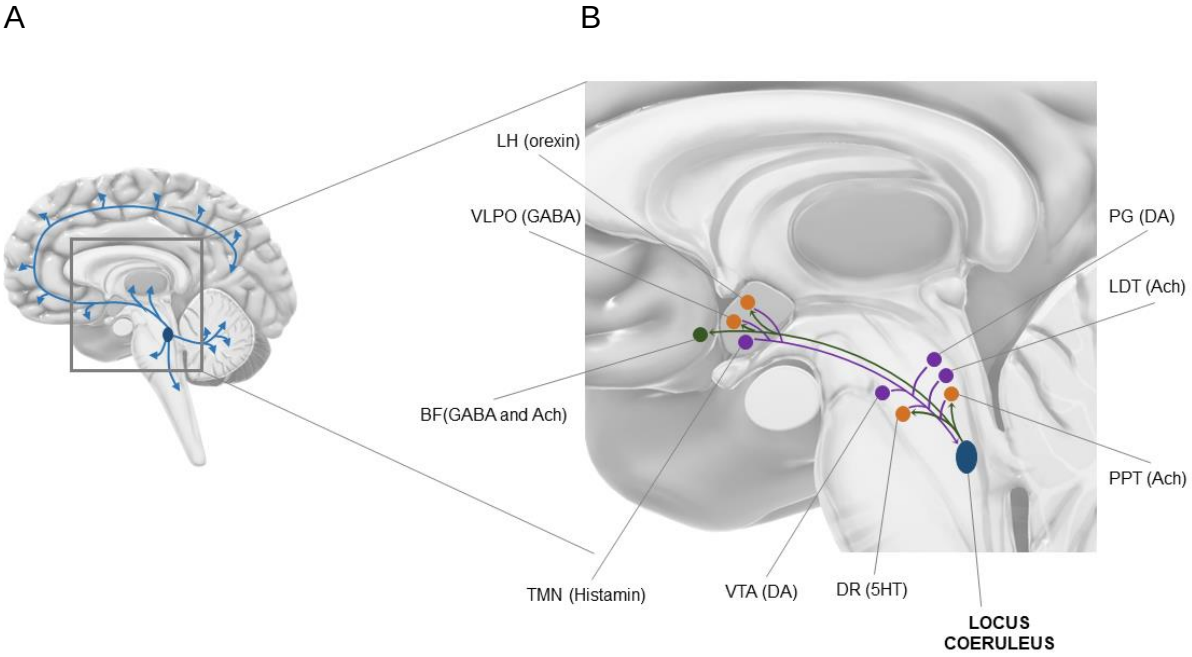

Figure 2.1. Afferences and efferences of the brainstem locus coeruleus (LC)

(A) LC sends ubiquitous projections over most of the brain including the hippocampus, amygdala, thalamus, and neocortex.

(B) LC relationships with other sleep-wake centers. Purple dots represent nuclei which send projections to the LC; green dots represent nuclei which receive inputs from the LC; orange dots represent nuclei which both send and receive projections from the LC. All nuclei are positioned for illustrative purpose and may not reflect their precise anatomical location.

LC efferences target cholinergic and GABAergic neurons of the basal forebrain (BF), GABAergic neurons of the ventrolateral preoptic area (VLPO) in the anterior hypothalamus, orexinergic neurons of the lateral hypothalamus (LH), serotoninergic neurons of the dorsal raphe (DR), and cholinergic neurons of the pedunculopontine tegmentum (PPT) nucleus. LC afferences arise from orexinergic neurons of the lateral hypothalamus (LH), GABAergic neurons of the VLPO, histaminergic neurons of the tuberomammillary nucleus (TMN), dopaminergic neurons of the ventral tegmental area (VTA), serotoninergic neurons of the dorsal raphe (DR), cholinergic neurons of the pedunculopontine tegmentum (PPT) and laterodorsal tegmentum (LDT), and the dopaminergic neurons of the periaqueductal grey matter (PG).

Likewise, afferent projections to LC-NE neurons were initially quantified with retrograde labeling techniques (Aston-Jones et al., 1991), and the picture was recently refined by viral tracing methods, showing that the LC receives connections from more than 100 brain regions (Schwarz et al., 2015). Among the sleep-wake circuitry, LC-NE neurons receive inputs from orexinergic neurons of the lateral hypothalamus, GABAergic neurons of the VLPO and ventral lateral hypothalamus, histaminergic neurons of the tuberomammillary nucleus (TMN), dopaminergic neurons of the VTA, serotoninergic neurons of the dorsal raphe, cholinergic neurons of the PPT and LDT, and dopaminergic neurons of the periaqueductal grey matter (Lew et al., 2021; Samuels and Szabadi, 2008; Saper and Fuller, 2017) (**Figure 2.1**).

Importantly, the existence of GABAergic LC neurons located in the dendritic field surrounding the LC nucleus (termed pericerulear or peri-LC region), and also found intertwined with LC-NE neurons, was recently identified as serving an important inhibiting function to locally regulate LC-NE tonic and phasic activity (Breton-Provencher and Sur, 2019).

This complex afferent-efferent organization, complemented by a local gain mechanism based on GABAergic inhibition of LC-NE activity, allows LC-NE neurons to integrate information from multiple sources and act as a broadcasting center for the whole brain, which constitutes a crucial feature to support the many roles of the LC over multiple timescales (Chandler et al., 2019). Particularly for sleep-wake regulation, the anatomical interconnections with several sleep- and wake-promoting nuclei place the LC as an important contributor to the onset and maintenance of sleep and wakefulness states, as well as to their associated behavioral and electrophysiological properties.

The LC-NE system and the sleep-wake cycle

Early electrophysiological, pharmacological, and lesion studies

Early electrophysiological studies on the activity of the LC-NE system across the sleep-wake cycle in rodents, cats, and monkeys, established that tonic LC activity progressively decreases when animals switch from engaged, behaviorally active states (~3Hz) to quieter, resting conditions (~1Hz), to slow wave sleep (< 1Hz) (Chu and Bloom, 1973; Foote et al., 1980). During sleep, LC-NE neurons were found to anticipate sleep-to-wake transitions, as they display bursts of activity in the seconds preceding spontaneous or sensory-evoked awakenings (Aston-Jones and Bloom, 1981). This state-dependent LC-NE neuronal discharge pattern was further

corroborated by reports showing that NE levels in the pons, amygdala, and hippocampus are high during wakefulness, lower during quiet wake, and lowest during sleep (Kalen et al., 1989; Shouse et al., 2000). Likewise, a novel experimental paradigm that allows to track pupil diameter across wakefulness and sleep states in rodents revealed that pupil size was progressively smaller when shifting from wakefulness to sleep, mirroring the gradual silencing of LC-NE neurons across behavioral states (Yüzgeç et al., 2018).

Accordingly, a series of pharmacological studies investigating the impact of modulating LC-NE activity on sleep-wake periods showed that injection of α_2-adrenergic receptor agonists into the LC area, such as clonidine or dexmedetomidine, or combined α_1- and β-adrenergic receptor blockade, suppressed LC-NE activity and induced dose-dependent sedative states in rats (Berridge and España, 2000; Correa-Sales et al., 1992; de Sarro et al., 1987). In contrast, activating LC-NE neurons through yohimbine, an α_2-adrenergic receptor antagonist, increased wakefulness (de Sarro et al., 1987).

Unlike pharmacological studies, early lesion studies in rodents, rabbits, and cats provided conflicting evidence about the consequences of LC damages on sleep-wake states, with some reporting increased wakefulness and reduced drowsiness (Braun and Pivik, 1981; Cespuglio et al., 1982) while others found acute suppressed wakefulness (Jones et al., 1973) or limited to no effect on time spent in wakefulness or sleep states (Caballero and De Andrés, 1986). Of important note, the coverage and accuracy of LC lesions in the aforementioned studies were inherently linked to the technique used (*e.g.* electrolytic-, neurotoxic-, cryo-lesion). Thus, these inconsistencies may be tied to the extent of LC injuries and to robust compensatory responses within surviving LC neurons that help sustain NE release and post-synaptic NE uptake, as long as no more than 90% of the LC is damaged (Berridge et al., 2012), a threshold that was usually not reached in those studies. More recently, specific lesioning of more than 95% of LC-NE neurons in rats did not affect the total duration of wakefulness per 24h (Blanco-Centurion et al., 2007; Gompf et al., 2010), but significantly compromised the promoting effect of exposure to a novel environment on the maintenance of wakefulness (Gompf et al., 2010).

Saper and colleagues proposed the so-called ‘flip-flop’ mechanism to describe the transitions between wakefulness and sleep (Saper et al., 2001). According to this model, two mutually inhibitory circuits are driving the onset and maintenance of wakefulness and sleep, forming a bi-stable switch which supports consolidated

periods and helps preventing intermediate states: wakefulness is considered to be driven mainly by the influences of monoaminergic neurons, *i.e.* LC-NE, serotoninergic neurons from raphe nuclei, histaminergic neurons from TMN, and cholinergic neurons of PPT and LDT, while sleep is promoted through the inhibitory action on this arousal circuit by GABAergic and galaninergic neurons located in the VLPO and median preoptic nuclei (Saper et al., 2010). Importantly, the development of novel methods covering pharmacogenetics, chemogenetics, and optogenetics, was instrumental in enabling new experimental manipulations aimed at unraveling the precise role of the LC-NE system within this framework.

Shedding new light on the Blue Spot

In 2010, Carter *et al.* demonstrated that photoinhibition of LC-NE neurons during the active period caused a reduction in time spent in wakefulness as well as an increase in wake-to-sleep transitions. Conversely, photoactivation of LC-NE neurons during the inactive period produced immediate sleep-to-wake transitions (Carter et al., 2010). Moreover, using a dual optogenetic approach, they further showed that photoactivation of orexinergic neurons of the lateral hypothalamus, which send dense projections to the LC (Horvath et al., 1999), concomitant with photoinhibition of LC-NE activity prevented sleep-to-wake transitions, while simultaneous photoactivation of both nuclei significantly increased the probability of awakenings (Carter et al., 2012). Hence, the LC-NE system also serves as a necessary and sufficient gateway for the effect of upstream arousal-regulating neuronal ensembles (Carter et al., 2013, 2012). More recently, the role of LC-NE activity in sleep-to-wake transitions was further expanded to awakenings triggered by external perturbators, and it was shown that the probability to transition from sleep to wakefulness in response to auditory stimuli was increased after photoactivation of LC-NE neurons and decreased after their photoinhibition in rats (Hayat et al., 2020). Altogether, these compelling optogenetic findings demonstrate that LC-NE activity is crucially involved in the regulation of both endogenous and sensory-evoked transitions from sleep to wake states (**Figure 2.2**).

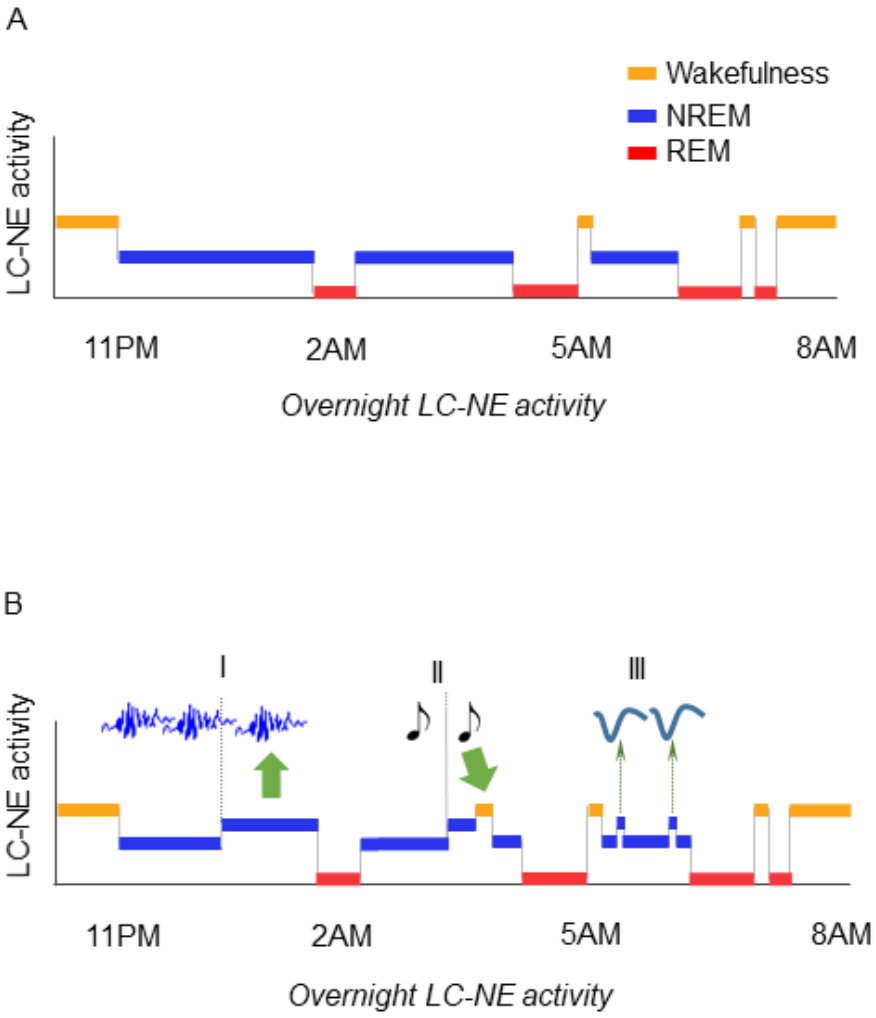

Figure 2.2. Overnight LC-NE activity vs. sleep macro- and microstructure.
(A) LC-NE activity and sleep macrostructure. LC-NE tonic firing rate is highest during wakefulness, lower during NREM sleep, and virtually silent during REM sleep.
(B) LC-NE activity and sleep microstructure. Elevated LC-NE activity (I) reduces spindles occurrence and (II) increases likelihood of sensory-evoked awakenings; (III) Learning-dependent increase in LC-NE activity is phase-locked to the rising phase of NREM slow oscillations. Separation into I/II/III segments is for visual representation only, as they are interchangeable with regards to their time of occurrence.
Figure overall layout is inspired by (Van Someren, 2021)

Importantly, while the LC consists mainly of NE-ergic neurons, other chemical compounds, including wake-promoting neurotransmitters such as dopamine, are produced or co-released by LC neurons (Oh et al., 2019b). A recent study therefore used optogenetics combined with LC-NE cell-type specific selective knockdown of dopamine beta hydroxylase, a necessary enzyme for NE synthesis: after this specific

genetic disruption of NE production in mice, the duration of wakefulness was reduced and optogenetically-driven sleep-to-wake transitions immediately following stimulations of LC-NE neurons were abolished (Yamaguchi et al., 2018), supporting the essential role of NE for LC-mediated regulation of wakefulness periods.

Additional influences: importance of circadian and homeostatic factors

It is well established that the organization of the sleep-wake cycle is regulated by overarching circadian ('process C') and homeostatic ('process S') factors (Borbély et al., 2016), and that alteration of these two processes in the course of human aging underlie the age-related changes in sleep and wake phenotypes (Mander et al., 2017). Crucially, a series of evidence suggest that the circadian and homeostatic processes may directly or indirectly involve the LC-NE system (Aston-Jones et al., 2007).

Trans-synaptic retrograde tracing revealed that the LC receives indirect input from the suprachiasmatic nucleus (SCN) of the hypothalamus, the central pacemaker responsible for the generation of circadian rhythms, through critical relay nuclei including the dorsomedial hypothalamic nucleus (DMH) (Aston-Jones et al., 2001). In that same study, Aston-Jones *et al.* reported that tonic LC-NE activity itself displays a certain degree of circadian variations: in rats maintained in free-running conditions under constant darkness for at least three days, LC-NE neurons were found to discharge faster during the active circadian period compared to the inactive circadian period. In addition, they showed that such circadian fluctuations in LC-NE activity was abolished after DMH lesioning (Aston-Jones et al., 2001). The existence of circadian rhythmicity within the LC-NE system was further corroborated by reports of circadian influences on NE content within the LC and SCN (Semba et al., 1984) and on the number of α- and β-receptors in the rat brain (Wirz-Justice et al., 1980), as well as circadian variations in steady-state pupil size (Daguet et al., 2019; Van Egroo et al., 2019a) and in glucose metabolism in the LC area (Buysse et al., 2004) in humans. Overall, these findings suggest that, as part of the SCN-DMH-LC circuit, the LC-NE system is under strong circadian influence and, in turn, contributes to the circadian regulation of the sleep-wake cycle (Szabadi, 2018).

With regards to homeostatic influences, the increase in slow wave sleep after sleep deprivation, which constitutes a gold standard marker of the wake-dependent build-up of sleep need, was largely dampened by lesioning the LC-NE system in rats (Cirelli et al., 2005; Cirelli and Tononi, 2004; González et al., 1996). Interestingly, release of NE during wakefulness strongly promotes synaptic potentiation (Tully and

Bolshakov, 2010), which has been directly related to the amount of slow-wave activity (0.5-4Hz) during the following night (Huber et al., 2007; Vyazovskiy et al., 2009). Moreover, *in vivo* microdialysis experiments in mice showed that NE levels in the prefrontal cortex increase during prolonged wakefulness, and that LC-NE neurons projecting to the medial prefrontal cortex were particularly affected by neural fatigue (Bellesi et al., 2016), which may contribute to the specific cognitive impairment resulting from sleep deprivation (*i.e.* vigilance, working memory).

The LC-NE system and sleep macrostructure

Akin to the discrepancy observed in LC-NE discharge rate between wakefulness and sleep, the activity of the LC-NE system is differentially regulated across sleep stages. In 1975, Hobson *et al.* described that transitions from NREM to REM sleep were anticipated by an increase in activity within a pool of cholinergic neurons located in the gigantocellular tegmental field (FTG), concomitant with a silencing of neurons from the posteroventral LC (Hobson et al., 1975). Therefore, the authors proposed a computational model revolving around a reciprocal inhibitory interplay between REM-OFF LC-NE and REM-ON cholinergic FTG neurons to regulate the onset and offset of REM episodes (McCarley and Hobson, 1975). Accordingly, early electrophysiological observations indicated that reduced, but existent tonic LC-NE activity occurred during NREM sleep, while LC-NE neurons were virtually silent during REM sleep (Aston-Jones and Bloom, 1981; Foote et al., 1980) (**Figure 2.2**). Likewise, REM sleep has been associated with the lowest level of extracellular NE in the amygdala and in the pons (Shouse et al., 2000) and with the highest degree of pupil constriction (Yüzgeç et al., 2018), reflecting almost complete inactivity of the LC-NE system.

As for the investigation of the sleep-wake cycle, conflicting evidence arose from lesion studies assessing the impact of damaging the LC on NREM and REM duration (Blanco-Centurion et al., 2007; Braun and Pivik, 1981; Caballero and De Andrés, 1986). In addition, neither optogenetic inhibition nor stimulation of LC-NE neurons yielded significant changes on the duration of REM episodes or the probability to transition from NREM to REM sleep (Carter et al., 2010). These latter observations led to the hypothesis that the LC-NE system would be involved in the modulation of REM sleep rather than directly contributing to its genesis (Saper et al., 2010; Saper and Fuller, 2017); a causal role that has now been principally attributed to mutually inhibitory cell groups located in the mesopontine tegmentum: the REM-ON glutamatergic and GABAergic cells of the sublaterodorsal tegmental nucleus (also

termed subcoeruleus region in humans) and the REM-OFF GABAergic neurons of the ventrolateral periaqueductal grey matter and lateral pontine tegmentum (Peever and Fuller, 2017). It is important to note, however, that the effort to identify the neurobiological circuit underlying REM sleep control is still ongoing, and the contributions of additional nuclei, are continuously being unveiled (Kroeger et al., 2019; Valencia Garcia et al., 2018).

The LC-NE system during REM sleep: a silence that speaks volume

The complete silencing of LC-NE neurons observed during REM sleep episodes is a unique condition for the brain (Van Someren, 2020). Among the many purposes attributed to REM sleep, it is therefore understandable that some of them directly relate to the (dys)function of the LC-NE system (Peever and Fuller, 2017). For instance, it was proposed that the temporary NE-free milieu inherent to REM sleep constitutes a prerequisite for the upregulation of NE receptors after the continuous exposure to NE during wakefulness and NREM sleep (Siegel and Rogawski, 1988). Others proposed that the silence of LC-NE neurons during REM sleep acts as a critical process to maintain an optimal level of brain excitability, based on a series of observations demonstrating that elevated concentrations of NE follow specific REM sleep deprivation and that the resulting excess of NE induces aberrant neuronal excitability through modulation of Na-K ATPase activity (Amar and Mallick, 2015; Khanday et al., 2016).

Importantly, the activity pattern of the LC-NE system during sleep has also been recently linked to the consolidation of memories (Poe, 2017; Sara, 2017), with a particular emphasis on the sleep-dependent processing of emotional memories (Goldstein and Walker, 2014). Within that framework, the absence of LC-NE neuronal activity during REM sleep is proposed to provide synapses with a suitable neuromodulatory environment that allows for neuronal depotentiation, which is otherwise blocked by the effect of NE, and subsequent plastic rewiring of memory schemas in the hippocampus (Poe, 2017), while the timely discharge of LC-NE neurons during NREM sleep would promote plasticity during memory replay (discussed in more details in the following section) (Sara, 2015). With regards to consolidation of emotional memories, LC-NE neuronal silence during REM episodes is thought to support the integration of the content of a given emotional event in memory networks while disconnecting and downplaying its associated arousal in limbic structures, so that the event can be subsequently recalled without triggering the original emotional reaction (Van Someren, 2020). Particular attention has

therefore been allocated towards elucidating the emotional function of LC-NE silence during REM sleep, as its disruption –or an endogenous predisposition to be disrupted– has been further postulated to strongly contribute to certain psychiatric conditions, such as post-traumatic stress disorder (PTSD) (Vanderheyden et al., 2014) and insomnia (Van Someren, 2020). Correspondingly, novel experimental findings in humans showed that the sleep-dependent adaptation to stressful stimuli or self-conscious emotions is impaired after restless REM episodes, reflecting abnormal activity of the LC-NE system during REM sleep, in healthy individuals and insomnia patients (Gong et al., 2021; Wassing et al., 2019a) as well as in PTSD (Lipinska and Thomas, 2019).

Altogether, these elements provide a strong rationale to further investigate the causes, correlates, and consequences of abnormal LC-NE activity during critical time windows of supposed silence, especially given the foreseeable clinical applications among several LC-NE-associated psychiatric and neurological disorders, but also in non-pathological aging which typically involves an increase in time spent in lighter sleep stages at the expense of REM sleep.

The LC-NE system and sleep microstructure

While investigating LC-NE unit activity across the sleep-wake cycle, Aston-Jones & Bloom already noted that, during NREM sleep, tonic LC-NE activity displayed consistent fluctuations around spindles (trains of transient 12-16Hz waves): LC-NE neurons became almost silent in the seconds preceding the onset of a spindle, substantially increased firing during spindle activity, and decreased discharge again after the spindle offset (Aston-Jones and Bloom, 1981). These findings were recently refined and expanded by fiber photometry and optogenetic studies in rodents demonstrating that troughs in extracellular NE concentration during NREM sleep concurred with spindle activity (Kjaerby et al., 2020; Osorio-Forero et al., 2021), and that stimulation of LC-NE neurons reduced spindle occurrence (Hayat et al., 2020; Osorio-Forero et al., 2021) (**Figure 2.2**) and impaired sleep-dependent memory consolidation (Novitskaya et al., 2016; Swift et al., 2018), while their inhibition increased spindle density and interfered with their temporal distribution, specifically through altered NE signaling in the thalamus (Osorio-Forero et al., 2021). In addition to the interplay with spindle activity, electrophysiological recordings in rats further showed that LC-NE neurons display a learning-dependent increase in activity during NREM sleep (Eschenko and Sara, 2008), and that this activity is phase-locked to the rising phase of NREM cortical slow oscillations (Eschenko et al., 2012) (**Figure 2.2**).

Overall, these findings point at an overarching function for LC-NE neurons to provide memory circuits with an optimal neuromodulatory background; a dual task which involves balancing between promoting synaptic potentiation during NREM sleep and synaptic depotentiation during REM sleep (Van Someren, 2020). Importantly, the interplay between LC-NE activity and sleep microstructure might be particularly relevant in human aging, as the preservation of those microstructural aspects have been linked to preserved cognitive performance in older adults (Djonlagic et al., 2020).

The LC-NE system and sleep-wake disruption in human aging, AD and ID

The vast majority of the findings described so far are based on animal studies, which provide inherent advantages when characterizing the consequences of experimental manipulation of LC-NE neurons on wakefulness and sleep. However, early evidence from human studies also contributed to identify and characterize the important role of the LC-NE system for sleep-wake regulation mechanisms. Almost 50 years ago, studies in Parkinson's disease or progressive supranuclear palsy (PSP) patients suggested that damage to the LC may underlie the observed disruption of wakefulness and sleep periods, including alteration of EEG features across sleep stages, increased nocturnal awakenings, and suppression of REM episodes (Gross et al., 1978; Mouret, 1975). A single case study in a young adult with cerebral palsy further reported that electrical stimulation of the LC led to an increase of wakefulness at the expense of REM sleep (Kaitin et al., 1986). Although limited, these initial observations hint at the translational potential of animal findings and support the relevance of investigating LC-NE-mediated sleep-wake alterations in conditions associated with LC injuries in humans. Here, we argue that Alzheimer's disease (AD) provides a valuable research perspective, given that the LC-NE system holds a crucial position by being at the intersection of initial AD-related pathophysiological processes (Braak et al., 2012) and sleep-wake dysregulation. At the same time, a recent theoretical model of insomnia disorder (ID) suggests that the LC is a potential critical node of a functional network, also comprised of salience and limbic networks, that can lead to disrupted overnight emotional adaptation following a cascade of neuronal events and constitute the neuronal ground of ID. In this final section, we will gather evidence supporting that the LC-NE system constitutes a strong neurobiological candidate underlying the sleep-wake disturbances, including ID, and commonly observed along the progression of AD.

Sleep-wake regulation and AD pathogenesis

As illustrated by a growing body of evidence over the past decade, sleep-wake dysregulation has emerged as a potent modifiable factor to slow down the characteristic pathophysiological processes of the disease, *i.e.* the accumulation of beta-amyloid (Aβ) and tau misfolded proteins together with neurodegeneration, as early as during the preclinical stages of the disease (Van Egroo et al., 2019c). Indeed, important discoveries established that the disruption of sleep and wakefulness constitutes a core mechanism of early AD pathogenesis: the physiological dynamics of both Aβ and tau proteins, encompassing their release and clearance are regulated by the sleep-wake cycle (J. K. Holth et al., 2019; Xie et al., 2013; Yamada et al., 2014). Conversely, both Aβ and tau pathology *per se* induce alteration of the sleep-wake cycle in AD mouse models (Holth et al., 2017; Jee Hoon Roh et al., 2012), supporting bidirectionality in the relationships between sleep-wake disturbances and AD-related neuropathological processes (Van Egroo et al., 2019c).

Crucially, landmark *post-mortem* studies in humans revealed that, beside its close connection with sleep-wake regulation described throughout this review, the LC is among the first sites of tau pathology across the lifespan (Braak et al., 2012), such that the vast majority of individuals harbor abnormally phosphorylated tau in the LC by the age of 40 (Braak et al., 2011a). In addition, the consequences of accumulated tau burden within the LC were found to be specifically expressed in AD cases compared to other tauopathies (*i.e.* PSP, cortico-basal degeneration), as substantial LC neuronal loss appeared as a phenotype exclusive to AD (Oh et al., 2019a).

Combined, these observations lend support to a theoretical framework in which the LC-NE system would constitute a bridge connecting sleep-wake dysregulation and initial AD-related pathophysiological processes (**Figure 2.3**). In mice, chronic sleep disruption or intermittent short sleep for three days a week during one month in mice was sufficient to produce profound alterations in LC-NE morphology, as evidenced by a drastic reduction of LC-NE neuronal counts and axonal projections (Zhu et al., 2016). Moreover, in a mouse model of tauopathy, repeated exposures to shortened sleep accelerated tau accumulation within LC-NE neurons and its progression to the entorhinal cortex, hippocampus, and amygdala, and advanced the onset of neurobehavioral deficits (Zhu et al., 2018). Remarkably, these effects were long-lasting, with structural alteration of the LC-NE system and cognitive impairments persisting one year after the chronic sleep disruption protocol (Owen et al., 2021). These animal findings suggest that the negative consequences of early-in-life sleep-

wake dysregulation precipitate and sustain AD-related processes within the LC-NE system.

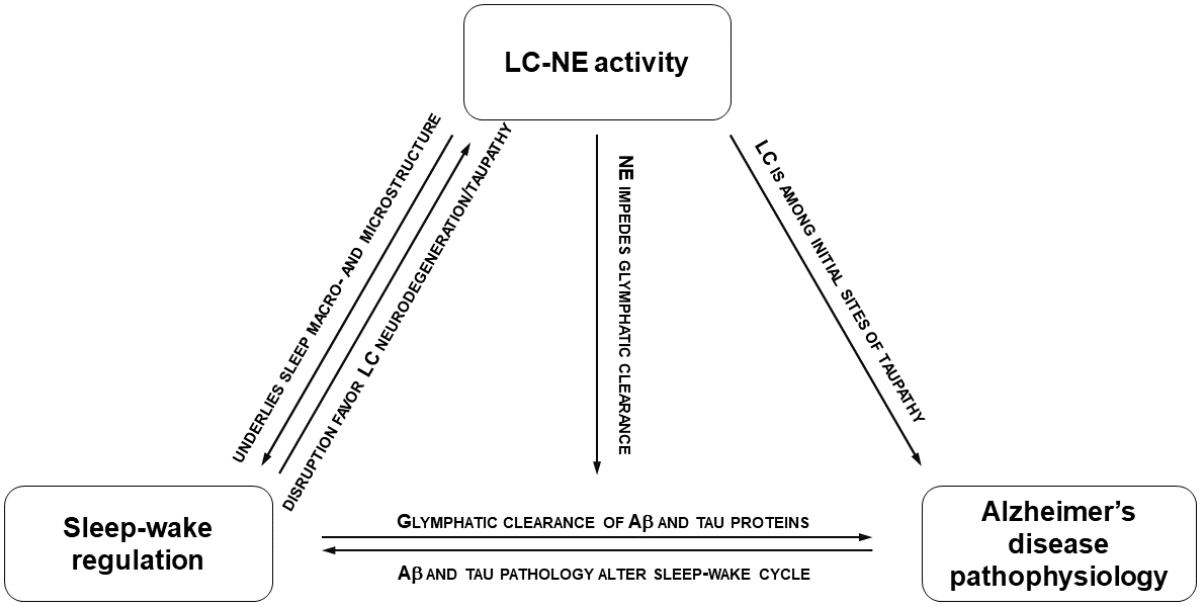

Figure 2.3. Schematic representation of the LC-NE system constituting a bridge that connects sleep-wake dysregulation and initial AD-related pathophysiological processes.

Bringing similar research questions into human studies has long been hindered by the considerable challenge to image the LC-NE, due to its deep location and its small size. However, recent advances in MRI methods now allow to investigate LC-NE structural and functional integrity with relatively short acquisition times to accommodate clinical studies (Kelberman et al., 2020). Importantly, the benefits of such sequences are exponentially amplified when implemented at ultra-high field (i.e. ≥ 7 Tesla MRI), given the sub-millimeter resolution available to identify and characterize the LC (Priovoulos et al., 2018). In that context, it was established that MRI-assessed LC-related contrast, supposedly reflecting neuronal and fiber projection density (Priovoulos et al., 2020), starts declining slowly in the 5th decade of life in cognitively unimpaired older individuals (Liu et al., 2017), while it is markedly reduced and correlated with Aβ pathology in AD patients (Betts et al., 2019) and with tau pathology in asymptomatic older individuals (Jacobs et al., 2021).

Interestingly, a parallel may be drawn between deterioration of the LC-NE system and the unfolding of sleep-wake disruption in late life; a relationship that appears

even more striking during the course of AD. Sleep disturbances are common in AD patients, starting as early as in the preclinical stages under the form of exacerbated age-related sleep-wake disruption (Van Egroo et al., 2019c), but their prevalence and magnitude strongly increase with disease severity (Gagnon et al., 2019) which often precipitate institutionalization (Peter-Derex et al., 2015). Of note, most of these disturbances pertain to sleep-wake dimensions that have been described so far as tightly linked to the function of LC-NE system based on evidence from animal models: fragmented sleep with more frequent nocturnal arousals and awakenings, insomnia, poorer REM sleep integrity, and reduction in spindle density and slow-wave activity (Gagnon et al., 2019). Yet, while the significant degeneration of the LC-NE system observed at histological investigation of AD brains was suggested to contribute to the disruption of the sleep-wake cycle commonly experienced by AD patients (Oh et al., 2019a), no direct assessments of sleep-wake measures were available in these post-mortem studies. Similarly, not many in vivo studies correlated LC-NE properties with sleep-wake measurements in healthy aging or AD, leaving important gaps in the understanding of the interplay between alteration of the LC-NE system, sleep-wake dysregulation, and AD-related pathophysiological processes in humans.

The role of the LC-NE system in insomnia disorder

Besides having a key role in sleep, the LC has been proposed to be at the core of vulnerability to insomnia disorder (ID): a model suggests the involvement of the LC-NE system in promoting insomnia not only by triggering accumulation of arousal but also via insufficient LC silencing during REM sleep, which alters synaptic plasticity in limbic circuits and resulting in altered overnight neuronal memory trace adaptation (Van Someren, 2021). More specifically, the LC-NE system is involved in the regulation of arousal, and its over-activity would increase the level of arousal and enhance the emotional reactions to stress, which will lead to a series of symptoms of hyperexcitability (Berridge, 2008; Yamamoto et al., 2014) and could contribute to an inability to fall or stay asleep associated with insomnia (Berridge et al., 2012).

Furthermore, the neuronal pathways of insomnia are not well defined due to a number of factors: the heterogeneity of insomnia symptoms, the lack of a phenotype that is objectively characterized and the descrepancy beween objective PSG sleep measures and self-reports. In addition, insomnia has not lent itself well to the development of animal models, and the absence of molecular or genetic biomarkers makes in vitro models difficult (Buysse et al., 2011). However, according to the

above-mentioned model, the contribution of diverse neuronal circuits into the final common path of insomnia may be restricted to an initial vulnerability to have insufficient LC silencing during REM sleep. This vulnerability, or sensitivity to input from the salience network and related circuits, is driven both genetically and environmenatally (early life adversity) (Van Someren, 2021). The *restless* REM sleep being characteristic of ID (Feige et al., 2008; Riemann et al., 2012) contains a high number of phasic events, like arousals and eye movements (Wassing et al., 2016)) and indicates insufficient LC silencing. This absence of NE-free timewindow results in disrupted balance between synaptic potentiation / depotentiation during REM sleep and in deficient synaptic plasticity of emotional / limbic circuits in the brain.

This model is theoretical and opens up broad perspective for research targeting the role of the LC-NE system in ID. Recently there have been two studies investigating the LC functional connectivity in insomnia. One of them showed that the enhanced functional connectivity between the LC and various brain regions including those belonging the default mode network, which plays important role in the maintenance of consciousness and the right superior orbitofrontal cortex, a part of the prefrontal cortex, which through ascending projections in LC-NE system is involved in attentional and cognitive control (Li et al., 2022). Another study showed that abnormal functional connectivity between right LC and left dorsal anterior cingulate cortex, a part of executive control network, was associated with the anxiety scores in insomnia (Gong et al., 2021).

Conclusion

A colossal number of previous reports and meta-analyses indicate close relationship between sleep problems including ID, aging and AD. This chapter summarizes evidence that the noradrenergic LC activity is a strong candidate to play critical roles in the mutual relationship between sleep-wake dysregulation and AD, as well as in ID. Many questions remain unanswered, however, with regards to the corresponding mechanisms that may take place long time before the onset of these diseases, and the exact contribution of the LC-NE system to the regulation of sleep and wakefulness and their associated features, especially in humans as illustrated by the scarce number of *in vivo* human studies. In the experimental Chapters 3-5 we will address such questions, and in the Discussion we will propose our understanding of the results and research perspectives for further investigation of these mechanisms.

Study objectives

As we showed in the introduction, sleep alterations constitute risk factors or potential markers of the future development of diseases including ID and AD. These diseases are characterized by relatively long preclinical periods during which sleep could either contribute to neuropathology or be altered by neuropathological processes. The LC may be one of the first brain areas whose integrity and functioning contribute to the initiation and progression of these disorders, and its role in human sleep remains understudied. FMRI scanning together with EEG recordings during sleep in patients and controls could supply valuable data for inspection of how state-dependent LC activity is related to sleep and disease. This is a difficult goal, though, as it would be nearly impossible to sleep through the night in the scanner (and with EEG), especially taking into account that there are certain sleep and health peculiarities in both ID and AD which would impede the execution of such protocol. In addition, several factors, including comorbidities, lifetime environmental exposure and aging, may hamper isolation of key sleep aspects.

We therefore decided to employ genetics as a proxy measure for AD and ID in young healthy adults when seeking associations between sleep and AD/ID. Furthermore, following the assumption that intrinsic LC deterioration / malfunction that would be reflected in wakefulness and sleep can be captured by the MRI scanner during an attentional activity, we used this LC task-related activity as a proxy measure of the LC functional integrity. These two approximations did not nevertheless threaten the validity of our findings, but rather gave us room for some fruitful speculation in the discussion. The general objective of this dissertation was to explore healthy sleep as well as disease- and age-related sleep alterations in humans. Three studies are included in this work, of which in the first two we looked into the genetic predisposition for AD and ID in their relation to healthy sleep in youth. In the third study we investigated the role of the LC in shaping human sleep in healthy younger and older individuals, and we link it to ID and AD in the discussion. Hereafter I describe the goals of each study in more detail.

In the first study (Chapter 3) we took advantage of the possibility to gather genetic variants into a polygenic risk score (PRS), based on the whole-genome single nucleotide polymorphisms (SNPs) (Dudbridge, 2013). PRS allows the assessment of individual genetic predisposition for AD decades before its onset. Our goal was to see whether PRS for AD may be associated with sleep phenotypes in young adults without any AD symptoms. We computed whole-genome PRS for AD and phenotyped sleep under different sleep conditions, including baseline sleep, recovery

sleep following sleep deprivation, and extended sleep opportunity, in a sample of 363 healthy young men. We hypothesized that the sleep features of interest would be correlated with the genetic risk for AD; the results would contribute to understanding of common mechanisms between sleep and AD.

In the second study (Chapter 4), using the same approach with PRS, we looked at sleep characteristics related to ID in healthy young people (N = 461). There has been plenty of research seeking for differences in sleep measures in ID patients and healthy controls, and meta-analyses revealed that the most prominent contrast is found in sleep continuity (Baglioni et al., 2014), including higher number of nocturnal awakenings and arousals and consequently reduced total sleep time, as well as spectral characteristics of NREM and REM sleep (Riemann et al., 2012). We tested whether, in healthy people without sleep complaints but having higher genetic predisposition for ID, sleep may be already altered. For this purpose, we computed again individual PRS but to ID. We also extracted sleep characteristics from EEG nocturnal sleep recordings. We then looked at the sleep traits and their relation to ID PRS. ID very often starts and progresses prior or together with comorbid conditions affecting the central nervous system, such as anxiety and depression and its prevalence is high in aging when it constitutes a risk factor for neurodegenerative diseases. We wanted to unravel part of the core sleep traits which might constitute the sleep phenotype with a higher risk of ID. The hypothesis was that macro- and micro-structural objective sleep characteristics as measured with electroencephalography (EEG) which are present in insomnia-like sleep, would be associated with higher genetic risk for ID as assessed by PRS.

In the third chapter we aimed at exploring neuronal mechanisms underlying sleep variability in younger (N = 33; 18-35 y.o.) and older (N = 19, 50-70 y.o.) populations. The study was centered on the LC and its contribution to sleep and wakefulness. It is established that the LC is involved in regulating diverse brain functions, including arousal, attention and sleep, but the exact mechanism is not known, and most of the research had been done in animal models. We wanted to assess in humans if the LC functioning during wakefulness is associated with sleep, with the assumption that the LC functional integrity affecting both wakefulness and sleep may be captured by functional magnetic resonance imaging (fMRI). We probed the LC activity through an attentional task which is known to elicit a robust LC response. We obtained individual LC activity by taking average beta estimates from functional activation maps during the oddball task inside individually segmented LC masks. We also recorded sleep

EEG during a night in the laboratory and extracted sleep features which included some of the most canonical sleep characteristics. We hypothesized that the functional response of the LC in the oddball task would be associated with the sleep features of interest which would provide direct evidence in humans that the LC functioning contributes to sleep variability.

Experimental results

Chapter 3: Alzheimer's disease risk and sleep phenotypes in healthy young men: association with more slow waves and daytime sleepiness

This results section has been published in *Sleep* in 2021. Supplementary materials related to this paper are displayed in **Appendix 2.**

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Abstract

Study Objectives. Sleep disturbances and genetic variants have been identified as risk factors for Alzheimer's disease. Our goal was to assess whether genome-wide polygenic risk scores (PRS) for AD associate with sleep phenotypes in young adults, decades before typical AD symptom onset.

Methods. We computed whole-genome Polygenic Risk Scores (PRS) for AD and extensively phenotyped sleep under different sleep conditions, including baseline sleep, recovery sleep following sleep deprivation and extended sleep opportunity, in a carefully selected homogenous sample of healthy 363 young men (22.1 y ± 2.7) devoid of sleep and cognitive disorders.

Results. AD PRS was associated with more slow wave energy, i.e. the cumulated power in the 0.5-4 Hz EEG band, a marker of sleep need, during habitual sleep and following sleep loss, and potentially with large slow wave sleep rebound following sleep deprivation. Furthermore, higher AD PRS was correlated with higher habitual daytime sleepiness.

Conclusions. These results imply that sleep features may be associated with AD liability in young adults, when current AD biomarkers are typically negative, and the notion that quantifying sleep alterations may be useful in assessing the risk for developing AD.

Introduction

Defective proteostasis of brain amyloid-beta (Aβ) and tau protein antedates the clinical manifestations of Alzheimer's disease by decades (Jack et al., 2018; Musiek and Holtzman, 2015; Scheltens et al., 2016). This so-called "preclinical" window constitutes an opportunity for internvention that would hopefully reduce the predicted increase in AD prevalence (Norton et al., 2014), despite the absence of disease modifying treatments in the foreseeable future. In this respect, the further identification of AD risk factors is of paramount importance.

AD patients can become restless at night and sleepy during daytime while their entire sleep-wake cycle becomes fragmented and disorganized (Van Egroo et al., 2019c). Critically, similarly to Rapid Eye Movement (REM) sleep behavioral disorder (RBD) in Parkinson's disease (Al-Qassabi et al., 2017), altered sleep has recently been related to increased risk for developing AD, over and above sleep disturbances in AD patients (Van Egroo et al., 2019c). Longer latency to fall asleep and reduced sleep slow waves and rapid eye movement (REM) sleep are associated with both Aβ plaques and Tau neurofibrillary tangles (NFTs) in cognitively normal participants (Branger et al., 2016; Lucey et al., 2019; Mander et al., 2015). Sleep fragmentation and the reduction in REM sleep quantity in cognitively normal individuals aged >60 y predict the future risk of developing AD (Lim et al., 2013; Pase et al., 2017). Acute sleep deprivation (J. K. et al. Holth et al., 2019; Ooms et al., 2014), and experimentally induced reduction of sleep slow waves (Ju et al., 2017), increases cerebrospinal fluid (CSF) Aβ and Tau protein content.

In *post mortem* human brain tissues, the first signs of brain protein aggregation are identified in the locus coeruleus (LC), a brainstem nucleus essential to sleep regulation (Mather and Harley, 2016a), under the form of pretangles, consisting of phosphorylated Tau protein (Braak and Del Tredici, 2011). Critically, LC pretangles can be detected during adolescence, while by age 30, they can be detected in the majority of the population (> 90%) (Braak and Del Tredici, 2011). With age, Tau deposits increase in the brain in a stereotypical manner and are tightly associated with cognitive decline in overt 'clinical' AD (Braak and Del Tredici, 2011). Individual variations in these intrinsic properties should be reflected in brain function, including sleep, whether or not Tau aggregation has already occurred.

Sporadic AD, the most common form of AD in the general population, has an estimated heritability ranging between 58% to 79% (Ertekin-Taner, 2010; Gatz et al., 2006). Individual Polygenic Risk Scores (PRS) for AD can be computed based on

results of published Genome Wide Association Studies (GWAS). These PRS reflect part of the genetic liability for AD in any asymptomatic individual and, at the group level, can be associated with phenotypes of interest which are related to the (risk) pathways leading to AD (Euesden et al., 2015; Ge et al., 2019). Recent studies reported significant association between AD PRS and CSF Aβ content (Ge et al., 2018; Martiskainen et al., 2015), cortical thickness (Sabuncu et al., 2012), memory decline (Marden et al., 2016), and hippocampus volume (Foley et al., 2017; Ge et al., 2018; Mormino et al., 2016) in cognitively normal older adults (> 45 y) but, importantly, also in young adults (18 -35 y) (Mormino et al., 2016).

Here, we conducted a proof-of-concept study to establish that sleep can be related to AD risk in young adults, using PRS for AD. We phenotyped sleep under different conditions (baseline, sleep extension, recovery sleep after total sleep deprivation) in a homogenous sample of young healthy cognitively normal men without sleep disorders and computed individual PRS for AD. We hypothesized that high PRS would be associated with sleep metrics that had previously been associated with AD features in cognitively normal older adults. We further explored whether subjective assessments and behavioral correlates of sleep quality would be associated with PRS for AD.

Methods

This research was approved by the Ethics Committee of the Faculty of Medicine at the University of Liège, Belgium.

Participants

All participants signed an informed consent prior to their participation and received a financial compensation. Three hundred and sixty-four young healthy men (aged 18-31 years) were enrolled for the study after giving their written informed consent, and received a financial compensation. Exclusion criteria were as follows: Body Mass Index (BMI) > 27; psychiatric history or severe brain trauma; addiction, chronic medication affecting the central nervous system; smoking, excessive alcohol (> 14 units/week) or caffeine (> 3 cups/day) consumption; shift work in the past year; transmeridian travel in the past three months; moderate to severe subjective depression as measured by the Beck Depression Inventory (BDI) (A. T. Beck et al., 1988) (score > 19); poor sleep quality as assessed by the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) (score > 7). Participants with sleep apnea (apnea hypopnea index > 15/hour; 2017 American Academy of Sleep Medicine criteria,

version 2.4) were excluded based on an in-lab screening night of polysomnography. One participant, part of a twin pair, was excluded from the analyses so that the analyzed sample included 363 participants (**Table 3.1**). Some EEGs were missing/lost/not recorded due to technical issues that were detected *a posteriori* for three to five participants per nights of sleep considered in this manuscript. No individual had missing EEGs for more than one night of sleep so that all 363 individuals contributed to at least part of the analyses reported here. The Epworth Sleepiness Scale (Johns, 1991) was used to characterize daytime sleepiness but was not used for inclusion. While most participants scored normal values (≤ 11), 28 participants had scores ranging from 12 to 15, corresponding to moderate daytime sleepiness. Because of an initial error in automatic evaluation of computerized questionnaires, seven participants had PSQI scores higher than cut-off (scores of 8 or 9). No participants were, however, taking sleep medication. To avoid reducing sensitivity, these participants were included in all analyses but removing them did not change statistical outcomes. Furthermore, IQ was estimated in all participants using the Raven Progressive Matrices (John and Raven, 2003). One item or more was not responded to by a few participants so that IQ was available in 347 participants. Likewise, the screening questionnaire did not include a clear question about number of years of educations, but was rather asking about current occupation, so that education was available in 300 participants. Including IQ or education in our statistical models (hence, in a reduced set of subject) did not affect the statistical outputs of the results presented below.

Although available in our laboratory, Ab- and tau- PET scans were not conducted in participants: it was felt to be unethical to expose them to an irradiation while results would most likely be normal.

N	363
Sex	Men
Ethnicity	Caucasian
Age (y)	22.10 ± 2.73
Height (cm)	180.39 ± 6.70
Body mass index (BMI) (kg m^{-2})	22.15 ± 2.31
IQ*	123.88 ± 11.14
Education (y)**	13.33 ± 1.60
Mood	3.00 ± 3.48
Sleep quality	3.46 ± 1.76
Daytime sleepiness	5.94 ± 3.54
Chronotype	50.11 ± 8.25
Rest-activity Fragmentation (a.u.)	0.10 ± 0.03
Baseline sleep duration (min)	451 ± 41

Table 3.1.
Sample characteristics (mean ± *SD*)

Mood was estimated by the 21-item Beck Depression Inventory II (A. T. Beck et al., 1988); sleep quality by the PSQI (Buysse et al., 1989); daytime sleepiness by the ESS (Johns, 1991); chronotype by the Horne–Östberg questionnaire (Horne and Ostberg, 1976). IQ was estimated using Raven Progressive Matrices (John and Raven, 2003). Rest fragmentation (arbitrary units, a.u.) was estimated as the probability of transition from rest to activity during estimated sleep based on actigraphy data from the 3 weeks of imposed regular sleep (Hammad and Reyt, 2019; Lim et al., 2013).

*IQ was available for 347 participants.

†Number of years of education was available for 300 participants

Experimental Protocol

Individual sleep-wake history was strictly controlled: during the three weeks preceding the in-lab experiment, participants were instructed to follow a regular sleep schedule according to their habitual sleep timing (+/-30 min for the first 2 weeks; +/-15 min for the last week). Actigraphy data showed that included participants faithfully followed the assigned schedules.

Figure 3.1 provides an overview of the protocol. On Day 1, a urine drug test was performed (10-multipanel drug) before completing an adaptation night at habitual sleep/wake schedule during which a full polysomnography was recorded in order to screen for sleep related breathing disorders or periodic limb movements. On Day 2, participants left the lab with the instruction not to nap (checked with actigraphy). They

returned to the laboratory at the end of Day 2, completed a baseline night of sleep under EEG monitoring at habitual sleep/wake schedule and remained in the laboratory until Day 7 under constant CCTV. A 12h sleep extension night under EEG and centered around habitual sleep mid-point was initiated on Day 3, in complete darkness with the instruction to try to sleep as much as possible. Day 4 included a 4h afternoon nap under EEG recording (centred 1h after the mid-point between morning wake-up time and evening sleep time) further dissipated any residual sleep need. What we termed the "before" night was also initiated on Day 4. It consisted in 8h sleep opportunity starting at habitual sleep time. During Day 5 and 6, participants remained awake for 40 hours under constant routine (CR) conditions [dim light < 5 lux, semi-recumbent position, 19°C ± 1, regular isocaloric food intake] before initiating a 12h recuperation night from habitual sleep time until 4h after habitual wake time. Except during sleep (darkness – 0 lux) and constant routine protocol (dim light < 5 lux), participants were maintained in normal room light levels oscillating between 50 and 1000 lux depending on location and gaze. Analyses of "before" night, nap and sleep deprivation protocol will be reported elsewhere. The current study focusses on baseline, extension and recovery nights of sleep.

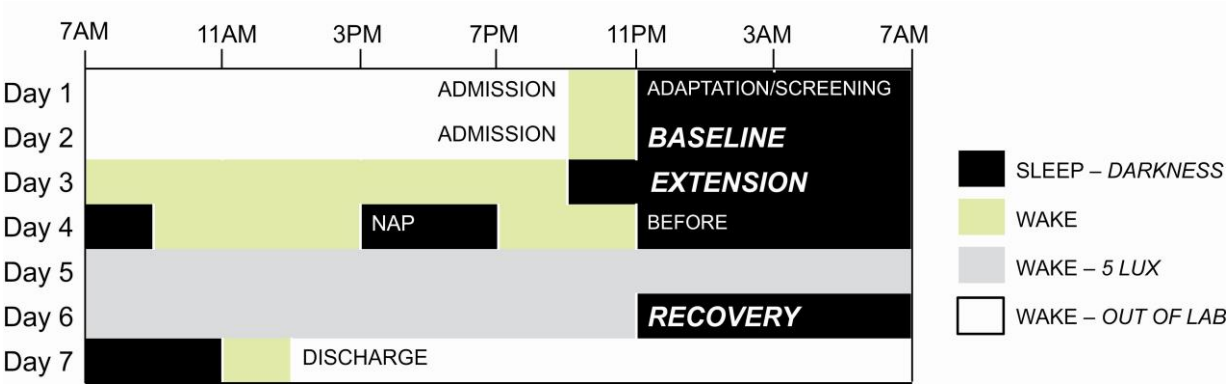

Figure 3.1. Overview of the protocol. Following 3 weeks of regular sleep at habitual times, 363 healthy young men aged ~22 years complete a 7-day protocol (displayed for a participant sleeping from 11 pm to 7 pm). Adaptation/screening and baseline nights were scheduled at habitual sleep-wake times. Extension nights consisted of a 12 h sleep opportunity centered around habitual sleep mid-point. Nap consisted of an afternoon 4 h sleep opportunity. The "before" (sleep deprivation) and recovery nights (from sleep deprivation) consisted of an 8 and 12 h sleep opportunity, respectively, all starting at habitual sleep time. Following the "before" night, volunteers completed a 40 h sleep deprivation protocol under strictly controlled CR conditions in dim light. Sleep periods included in the current analyses are in bold and italic.

EEG acquisitions and analyses

Sleep data were acquired using Vamp amplifiers (Brain Products, Germany). The electrode montage consisted of 10 EEG channels (F3, Fz, F4, C3, Cz, C4, Pz, O1, O2, A1; reference to right mastoid), 2 bipolar EOGs, 2 bipolar EMGs and 2 bipolar ECGs. Screening night of sleep also included respiration belts, oximeter and nasal flow, 2 electrodes on one leg, but included only Fz, C3, Cz, Pz, Oz and A1 channels. EEG data were re-referenced off-line to average mastoids. Scoring of sleep stages was performed automatically in 30-s epochs using a validated algorithm (ASEEGA, PHYSIP, Paris, France) (Berthomier et al., 2020) and according to 2017 American Academy of Sleep Medicine criteria, version 2.4. An automatic artefact detection algorithm with adapting thresholds (Wallant et al., 2016) was further applied on scored data. Power spectrum was computed for each channel using a Fourier transform on successive 4-s bins, overlapping by 2-s., resulting in a 0.25 Hz frequency resolution. The night was divided into 30 min periods, from sleep onset until lights on. For each 30 min period, slow wave energy (SWE) was computed as the sum of generated power in the delta band (0.5 – 4 Hz range) during all the NREM 2 (N2) and NREM 3 (N3) epochs of the given period, after adjusting for the number of N2 and N3 epochs to account for artefacted data (Skorucak et al., 2018). As the frontal regions are most sensitive to sleep-wake history (Schmidt et al., 2012), SWE was considered over the frontal electrodes (mean over F3, Fz, F4). To deal with the multiple comparison issue, we did not consider SWE over the other parts of the scalp (Dijk and Landolt, 2019). Additional analyses also considered cumulative power between 0.5 and 25 Hz during NREM and cumulative power between 2 to 6 Hz power during REM sleep as well through similar computation procedures.

Genotyping and Imputation

Blood sample were collected on Day 2 for DNA analyses. The genotyping was performed using the Infinium OmniExpress-24 BeadChip (Illumina, San Diego, CA) based on Human Build 37 (GRCh37). Missingness of the SNP markers were below 20% in all individuals. Using PLINK software (Purcell et al., 2007), we excluded the SNPs with a minor allele frequency (MAF) below 0.01, or Hardy-Weinberg disequilibrium (HWD) significance below 10^{-4}. Markers with ambiguous alleles (A-T, T-A, G-C, C-G) were excluded as well. We finally ended with 511,729 SNPs. To investigate the relatedness between the individuals, using PLINK --genome command, we computed the identity by descent (IBD) estimates for all pairs of

individuals. For 8 pairs, the composite pi-hat score was between 0.15 and 0.56 suggesting the existence of at least 3rd degree relatives in our cohort. We did not exclude any individuals at this level of analysis to keep the sample as large as possible, but removing one subject of each of these 8 pairs did not affect the statistical significance of any of the tests reported below. We merged our cohort with "1000 Genomes Project" (Altshuler et al., 2010) and employed principal component analyses (PCA) on the merged samples to see if our cohort was located in the European cluster (**Supplementary Figure 3.S1A**). We further assess allele frequencies coherence of our cohort with the European subset of "1000 Genomes Project" (**Supplementary Figure 3.S1B**). Markers with allele frequencies deviating more than 0.2 unit from European allele frequency were excluded (**Supplementary Figure 3.S1C**). Genotype imputation was performed using "Sanger imputation server" by choosing "Haplotype Reference Consortium (release 1.1)" (HRC) as Reference Panel and the Pre-phasing algorithm EAGLE2. Post-imputation QC was then performed very similarly to the one of above (MAF < 0.01, HWD < 10^{-4}, imputation quality score < 0.3). As a result of such filters, 7,554,592 variants remained for the analysis. However, to avoid having markers with allele frequencies deviating from European allele frequency, we computed the allele frequencies for the samples in our cohort after imputation and cross checked them with the European allele frequency (obtained from HRC Reference Consortium (release 1.1)) (**Supplementary Figure 3.S1D**). The markers whose allele frequencies were deviating more than 0.2 unit from European allele frequency were excluded.

Predicting Height

To validate common SNP assessments in our sample we predicted actual height based on Polygenic Scores computed based on a meta-analysis of a recent GWAS study (Yengo et al., 2018) on around 700,000 individuals. We used all the variants in the meta-analysis that were included in our cohort [3121 SNPS out of 3290]. The procedure for calculating the Liability for height is the same as the one described in the following section. **Supplementary Figures 3.S1E** visualize the Pearson correlation results between the actual values for Height and estimated genetic Liability of Height ($r = 0.46$, $p = 10^{-20}$). Explained variance is very close to that reported previously (Yengo et al., 2018), i.e. is 24.6% .

Polygenic Risk Score (PRS)

Polygenic risk score (PRS) is defined as the sum of multiple single-nucleotide polymorphism alleles associated with the trait for an individual, weighted by the

estimated effect sizes (Euesden et al., 2015; Ge et al., 2019). We used the estimated effect sizes from a GWAS by Marioni et al. (Marioni et al., 2018) which consisted of a meta-analyses of AD-by-proxy [UK Biobank data (Sudlow et al., 2015) - http://www.ukbiobank.ac.uk] and AD case-control data (Lambert et al., 2013) for a total of 388,324 individuals (67,614 cases – 25,580 patients and 42,034 self-reported parental history of AD – and 320,710 controls). Marioni et al. reported that the genetic correlation between AD-by-proxy and AD case-control was very high and not significantly different from 1, so that the genetic associations they computed, and therefore the PRS we computed based on their summary statistics, were truly dealing with AD.

The best p-value threshold that should be applied to AD case-control summary statistics is not established yet. Previous studies employed very exclusive GWAS p-values ($p \sim 10^{-8}$) (Sleegers et al., 2015) to more inclusive p-values (p = .5) (Escott-Price et al., 2015; Mormino et al., 2016), leading to the inclusion of effect sizes of a few tens to hundreds of thousands SNPs to compute AD PRS. Because we did not want to test all combinations of LD pruning and p-value thresholding, and then pick out the “best” one, we computed several PRS with different p-value thresholding and LD pruning combinations.

To generate a set of approximately independent SNPs in our sample, linkage disequlibrium (LD) clumping was performed using PLINK (Purcell et al., 2007) on window size of 1000-kb using a pairwise r^2 cut-off of 0.2 and a predetermined significance thresholds (*p*-value < 5 10^{-8}, 10^{-6}, 10^{-4}, 0.001, 0.01, 0.05, 0.1, 0.3, 0.5, and 1). Due to the effect of APOE in chromosome 19, we used a more stringent criteria pairwise r^2 cut-off of 0.01 for this chromosome. In addition, we also calculated the PRS using all the variants with no pruning, i.e. no correction for linkage disequilibrium, thereby selecting all SNPs for PRS construction. Although the later PRS was inevitably affected by complex LD structures, it was kept as one of the PRS. This procedure yielded 11 quantitative polygenic scores, under each significance threshold, for each individual in our cohort.

Height as a negative control

From the known and hypothesised biology, we did not expect any a priori association between the sleep phenotypes and a genetic liability for height. Therefore, we included an analysis of polygenic scores for height as a negative control, performing exactly the same association analyses as we did for liability to AD.

Actigraphy data collection and analysis

Actigraphy data were collected with Actiwatch 4 devices (Cambridge Neurotechnology ltd, UK) worn on the non-dominant arm. Data consisted in the sum of activity counts over 60-second intervals. Data were analyzed with *pyActigraphy* (Version v0.1) (Hammad and Reyt, 2019) which implements the computation of state transition probabilities from rest to activity (kRA) (Lim et al., 2013). In order to better reflect sleep fragmentation, this probability was calculated only over sleep periods for each study's participant. The sleep period is defined as the period comprised between the activity offset and onset times, derived from the average 24h activity profile. In addition, to mitigate the uncertainty on their exact timing, the offset and onset times were shifted by +15 min and -15min, respectively.

Statistical Analysis

We employed general linear model (GLM) to test the associations between sleep metrics of interests as a dependent variable and the estimated PRS as an independent variables and age, BMI and TST as covariates. Prior to the analysis, we removed the outliers among the sleep metrics by excluding the samples lying beyond 4 times the standard deviation (the final number of individuals included in each analyses is reported below each dependent variable in the supplementary tables). All analyses were performed in Python.

In this study, we analysed multiple traits and multiple polygenic risk scores (PRS) for association. To control the experiment-wise false positive rate, we estimated the number of independent tests that we performed, and set an experiment-wise p-value threshold accordingly. Since the traits are phenotypically correlated with each other and the PRSs are also correlated, we used the correlation structure to estimate the equivalent number of tests, which is the number of independent tests that would result in the same overall observed variation.

For each correlation matrix of traits and PRS, we performed a singular value decomposition (SVD), ordered the resulting eigenvalues and calculated the sum of all eigenvalues. We then calculated the minimum number of linear combination of the traits that resulted in 99% of the variation. For the 5 EEG phenotypic sleep traits this estimate was 5, showing that they are not highly correlated. Likewise, for the 3 non-EEG phenotypic sleep traits this estimate was 3. For the 11 PRS for AD and height, the resulting number was 8 and 4, respectively, consistent with a higher correlation structure among the multiple height predictors. Therefore, our analyses with the 5

EEG sleep metrics implies a total number of 40 and 20 tests when confronted to AD-PRS and height-PRS respectively. Hence, for any of our trait-PRS combination to be statistically significant when taken multiple testing into account, the p-value threshold are 0.00125 and 0.0025 for AD and height, respectively. Similarly, our analyses with SWE in recovery and extension nights and with SWE rebound, each imply 8 tests and a p-value threshold of p = 0.00625, while our analyses with 3 non-EEG sleep metrics 24 tests and a p-value threshold of p = 0.0021. Additional analyses compared lower and higher PRS quartile (i.e. 90 individuals with lowest AD PRS and 90 individuals with highest PRS) as well as APOE ε4 carriers vs. non carriers. For these analyses, groups were compared through t-tests.

We compute the minimum detectable effect size given our sample size. According to G-Power 3 (version 3.1.9.4) (Faul et al., 2007), taking into account a power of .8, an error rate α of 0.00625 (cf. above), with a sample size of 363, we were in a position to detect medium effect sizes r > 0.19 [confidence interval: 0.09-0.29] within a linear multiple regression framework including 7 predictors.

Results

Polygenic risk for AD is associated with the generation of slow waves during sleep

PRS were computed as the weighted sum of the effect sizes of the AD-associated SNPs, obtained from summary statistics of AD cases vs. controls GWAS (Euesden et al., 2015; Ge et al., 2019). PRS can indicate the presence of a genetic signal in moderate sample size studies (Euesden et al., 2015; Sabuncu et al., 2012) as long as it is computed based on a very large GWAS (Dudbridge, 2013; Santoro et al., 2018). We therefore used the summary statistics of one of the largest AD-GWAS available to date (N = 388,324) (Marioni et al., 2018) to compute individual PRS for AD in our sample and related these to sleep EEG characteristics following multiple quality control steps (cf. **Supplementary Figure 3.S1**).

We first focused on baseline sleep, as it is most representative of habitual sleep, to evaluate sleep metrics that might be associated with AD liability. Given our sample size, we reduced the multiple comparison burden by selecting *a priori* variables of interest among electrophysiology sleep metrics that have previously been related to Aβ and Tau in cognitively normal older adults: sleep onset latency [SOL] (Branger et al., 2016; Ettore et al., 2019), duration of wakefulness after sleep onset [WASO] (Ettore et al., 2019), duration of REM sleep (Pase et al., 2017), slow wave energy

[SWE] during NREM sleep (Lucey et al., 2019; Mander et al., 2015), i.e. the cumulated power in the 0.5-4 Hz EEG band, and hourly rate of micro-arousals during sleep (Ju et al., 2017). To compute PRS, one considers SNPs below a p-value threshold in the reference GWAS; the optimal threshold for SNP selection to best compute a PRS for AD is not established. To avoid bias in the threshold selection, we opted for computing PRS based on increasingly inclusive p-value thresholds (including SNPs reaching GWAS significance – $p < 5x10^{-8}$ - to very liberal $p < 1$), whilst also pruning SNPs based on their correlation structure (i.e. linkage disequilibrium) (**Supplementary Table 3.S1**) (Escott-Price et al., 2015; Mormino et al., 2016). In addition, we performed a PRS analysis using all SNPs without any selection.

General linear model (GLM) analyses controlling for age, body mass index (BMI) and total sleep time (TST), revealed an significant association between baseline night SWE and AD PRS ($p < 0.02$; **$\beta \geq 0.12$**) from a p-value threshold of p=0.05 up to selecting all SNPs; the association reached stringent experiment-wise correction for multiple comparisons when computing PRS using all SNPs, i.e. with potential linkage disequilibrium bias (see methods; **$\beta = 0.17$**; **Figure 3.2A; Supplementary Table 3.S2**). We performed a negative control analysis using a PRS for height, a variable for which no association with sleep metrics was expected, and found no association (**Supplementary Figure 3.S2A**). The association between AD PRS and SWE was positive (**Figure 3.2B**) indicating that higher SWE was associated with higher AD-PRS. SWE was also positively associated with TST (**Supplementary Table 3.S2**), which was expected since TST conditions the opportunity to generate slow waves, and negatively with age, which is in line with the literature (Carrier et al., 2011) but may still be surprising given the young age of our sample. Importantly, since GLM included TST and age, they are not driving the association we find between SWE and PRS for AD. Furthermore, we performed two additional analyses seeking for associations between PRS for AD and IQ or education, variable for which negative associations with AD pathophysiology were previously reported (Kunkle et al., 2019), and found no associations (**Supplementary Figure 3.S2B**). The link between PRS for AD and SWE may therefore more consistent (i.e. less variable) than the link between AD and IQ or education.

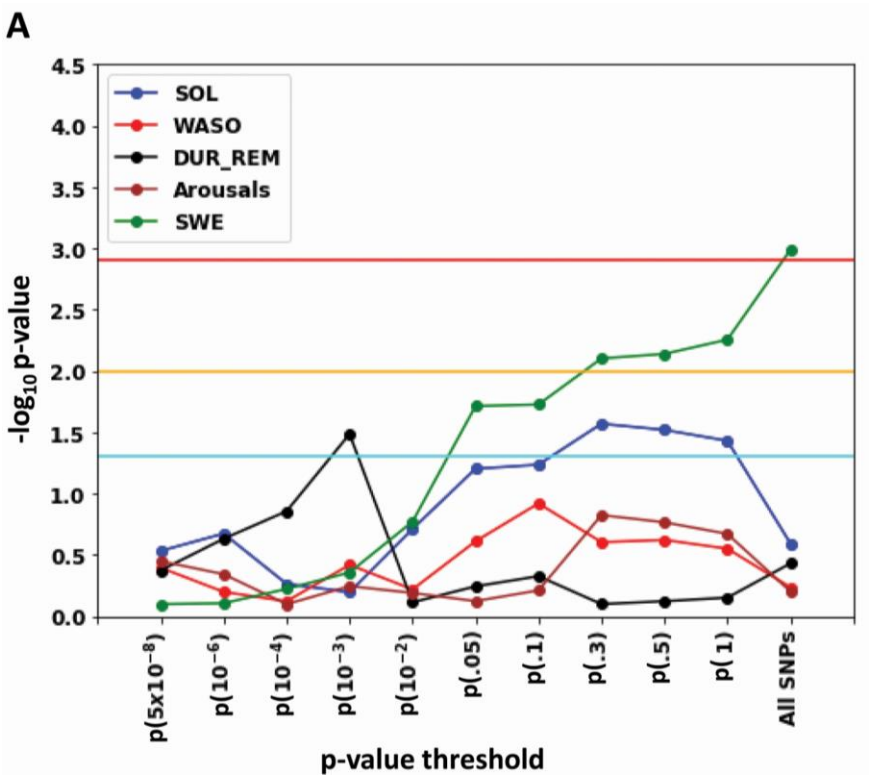

Figure 3.2A. Associations between PRS for AD and baseline night sleep metrics. (A) Statistical outcomes of GLMs with five sleep metrics of interest versus AD PRS from conservative ($p < 5 \times 10-8$) p value threshold to using all SNPs (N = 356). GLMs are corrected for age, BMI, and TST. The negative log transformation of p values of the associations is presented on the vertical axis. Horizontal lines in A and D indicate different p values thresholds: light blue = 0.05 (uncorrected); orange= 0.01 (corrected for five sleep metrics); red = 0.00125 (experiment-wise correction; see Methods).

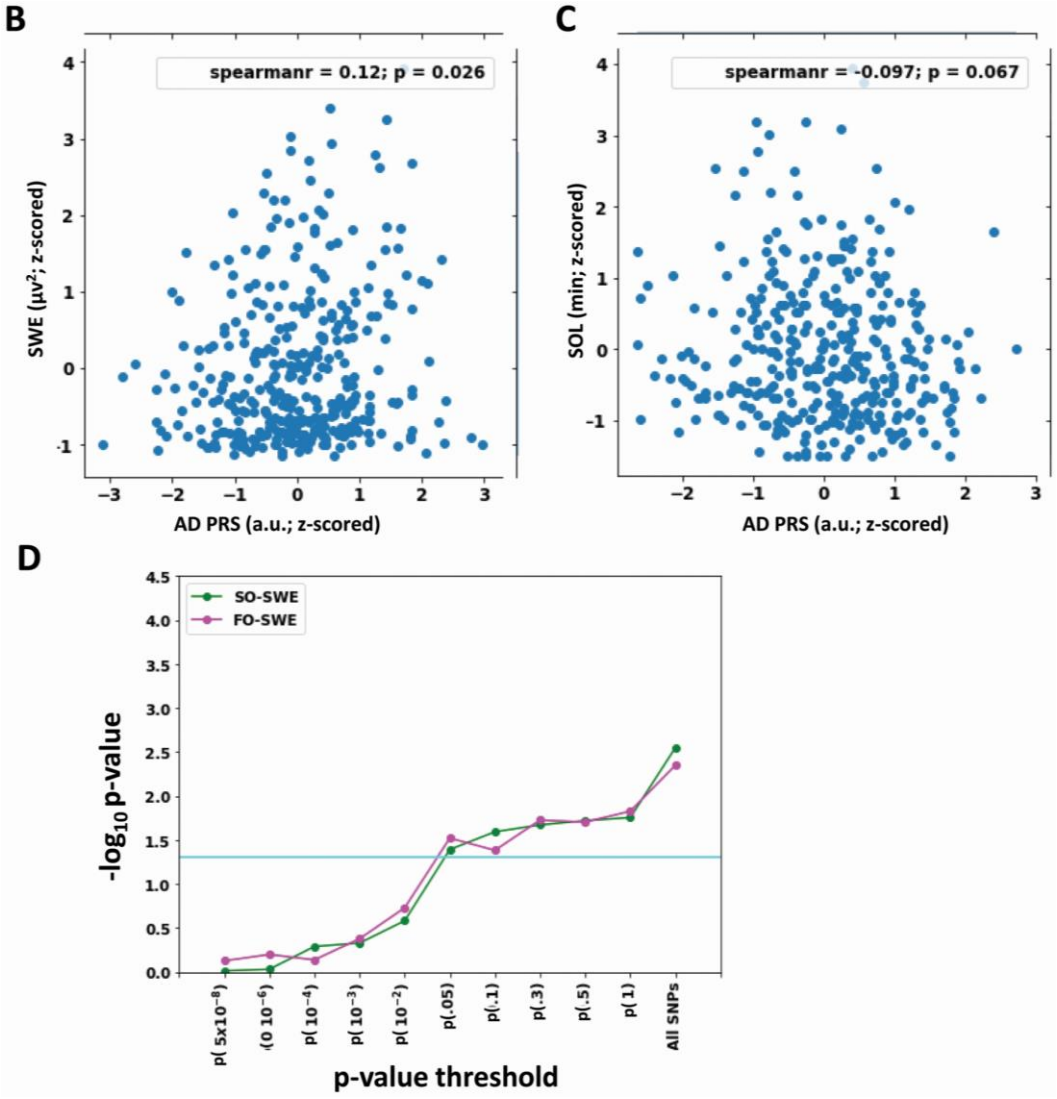

Figure 3.2 (B, C, D). Associations between PRS for AD and baseline night sleep metrics.

(B) The positive association between SWE during baseline night and AD PRS including all SNPs (N = 356). Spearman correlation r is reported for completeness (r = 0.12, p = 0.02), refer to main text Supplementary Table S2 for statistical outputs of GLMs. **(C)** The negative association between SOL during baseline night and AD PRS for p < 0.3. Spearman correlation r is reported for completeness (r = −0.11, p = 0.03), refer to main text Supplementary Table S2 for statistical outputs of GLMs (N = 356). **(D**) GLMs including SWE separated in the slower (SO-SWE; 0.5–1 Hz) and faster (FO-SWE; 1.25–4 Hz) frequency range from conservative p value thresholds to using all SNPs (N = 356). Horizontal blue line indicate p = 0.05 significance level. GLMs are corrected for age, BMI, and TST. Refer to Supplementary Table S3 for statistical outputs of GLMs. SOL, sleep onset latency; WASO, wake time after sleep onset; DUR_REM, duration of REM sleep; arousal, hourly rate of micro-arousals during sleep; SWE, slow-wave energy in NREM sleep (0.5–4 Hz).

Sleep onset latency (SOL) also reached significant association with AD PRS from a p-value threshold of p=0.05 up to p = 1 ($p \leq 0.04$; **$\beta = -0.11$**), but significance did not reach stringent experiment-wise correction for multiple comparisons (**Figure 3.2A; Supplementary Table 3.S2**). Hence, this result has to be considered with caution and will not be extensively commented upon. It is interesting to note, however, that the association between PRS for AD and SOL is negative, with higher PRS associating with shorter sleep latency (**Figure 3.2C**). Of note, REM% reached uncorrected significance ($p < 0.05$) for thresholding at p=0.05 (**$\beta = 0.1$**), with a positive association with AD PRS (**Figure 3.2A; Supplementary Table 3.S2**), but, since it is observed for only one p-value threshold, this will not be discussed any further.

These results indicate that, particularly when considering all SNPs to construct the AD PRS, the overnight power of the slow waves generated during Non-REM sleep, which is a widely accepted measure of sleep need (Dijk and Czeisler, 1995), is linearly and positively associated with AD genetic liability. This finding suggests that individuals with a higher genetic liability for AD have a higher need for sleep. This idea is further reinforced by the fact that association between SWE and AD PRS is also significant when only considering SWE of the first hour of sleep (Dijk and Czeisler, 1995) (**Supplementary Figure 3.S3 & Table 3.S3**), and the potential negative association with SOL, which depends in part on sleep need.

Since slow oscillations (SO), i.e. EEG slow waves < 1 Hz, may be distinct from faster slow waves (Steriade and Amzica, 1998), we further decomposed SWE into SO-SWE (0.5-1Hz) and faster-oscillations—SWE (FO-SWE; 1.25 – 4 Hz). Both SO-SWE and FO-SWE were similarly and significantly associated with AD PRS and for the same p-value thresholds (**Figure 3.2D; Supplementary Table 3.S3**). The association we found between SWE and AD PRS does not appear therefore to arise exclusively from either slower or faster slow waves.

Recovery sleep, slow wave sleep rebound and extension night

When considering sleep EEG of the other nights, we only included SWE, as it is the only sleep metric that was associated with PRS for AD at stringent correction for multiple comparisons threshold. Similarly to baseline night, when considering SWE during the recovery night that followed total sleep deprivation, GLM including age, BMI and TST, reveal that SWE and AD PRS are significantly associated ($p \leq 0.04$; **$\beta \geq 0.11$**) from p-value thresholding at p=0.1 up to using all SNPs (**Figure 3.3A; Supplementary Table 3.S4**), and the association reached stringent experiment-wise

correction for multiple comparisons at p-value threshold of p=1. Again, the association was positive with higher SWE associated with higher AD PRS (**Figure 3.3B**) and results were similar when considering only SWE of the first hour of sleep (**Supplementary Figure 3.S3 & Table 3.S4**). Individuals typically produce more sleep slow waves in response to sleep loss, as part of the homeostatic regulation of sleep (Klerman and Dijk, 2005). Therefore, individuals with higher need for sleep after sleep loss have a high PRS for AD.

Slow wave sleep rebound quantifies the physiological response to a lack of sleep based on the relative changes from normal sleep to recovery sleep following sleep loss. We computed the ratio between the initial SWE (1h of sleep) during recuperation and baseline nights to assess SWE rebound. GLM analysis, including age and BMI, indicated that SWE rebound reached significant association with AD PRS when including all SNPs (**β = -0.11**), but significance did not reach stringent experiment-wise correction for multiple comparisons (**Figure 3.3A; Supplementary Table 3.S4**). Sleep rebound is driven by sleep homeostasis which tightly regulates sleep duration and intensity based on prior sleep-wake history (Dijk and Landolt, 2019). Since we observe an association with AD PRS for a single p-value threshold at uncorrected p-value our findings suggest that, in our sample, AD PRS was not tightly associated with sleep homeostatic response. Interestingly though, Spearman's correlation indicated that SWE rebound was correlated to SWE during the recovery night ($r = 0.39$, $p < 10^{-14}$; **Figure 3.3C)**.

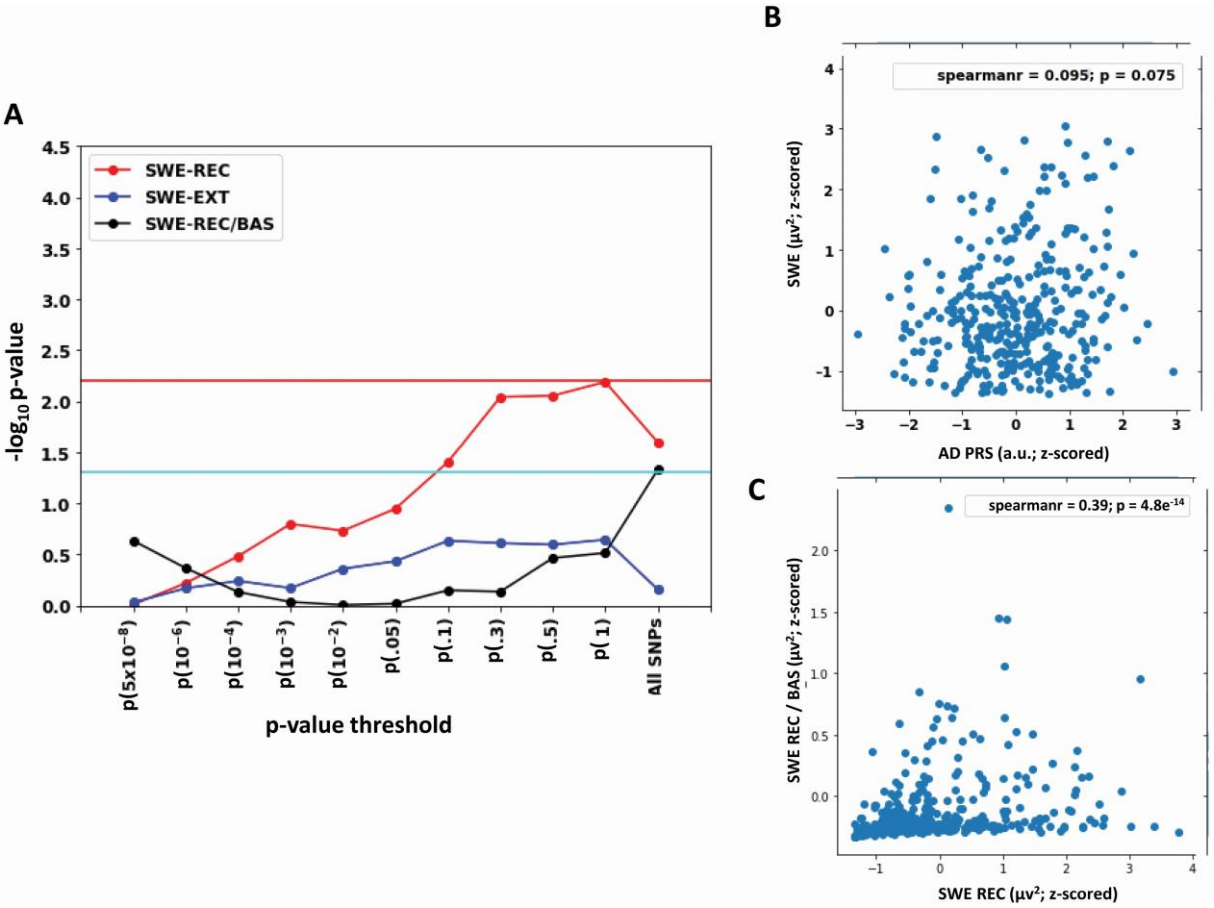

Figure 3.3. Associations between PRS for AD and SWE during recovery and extension nights and with SWE rebound. (A) Statistical outcomes of GLMs with SWE (0.5–4 Hz) in the recovery (REC; N = 353) and extension (EXT; N = 356) nights and with SWE rebound (REC/BAS; N = 344) versus AD PRS from conservative ($p < 5 \times 10-8$) to inclusive ($p < 1$) p value level and using all SNPs. SWE rebound consists in the ratio between SWE in the first hour of sleep of recovery and baseline nights. GLMs are corrected for age and BMI, and TST for REC and EXT. Negative log transformation of p values of the associations is presented on the vertical axis. Horizontal lines indicate different p values thresholds: light blue = 0.05 (uncorrected); red = 0.00625 (experiment-wise correction; see Methods). (B) The positive association between SWE during recovery night and AD PRS at $p < 1$. Spearman correlation r is reported for completeness (r = 0.01, p = 0.06) refer to main text Supplementary Table S4 for statistical outputs of GLMs (353). (C) Positive association between SWE during recovery and SWE rebound (SWE REC/BAS): Spearman correlation r = 0.36, $p < 0.001$ (N = 344).

We then considered SWE during the extension night and PRS for AD in a GLM, including age, BMI and TST. Results indicated that extension night SWE was not significantly linked to AD PRS. This may be because sleep timing for this particular night affects sleep quality (Dijk and Landolt, 2019; Dijk and Czeisler, 1995) (**Figure 3.3A**). In contrast to baseline and recuperation sleep periods which were initiated at habitual sleep time, sleep extension started 2 hours before habitual sleep time,

covering the end of a period known as the evening “wake-maintenance zone” corresponding to the time at which the circadian system maximally promotes wakefulness (Dijk and Czeisler, 1995). In addition, the circadian system is known to affect the relative content in Non-REM and REM sleep as well as in different EEG frequencies (Dijk and Landolt, 2019; Dijk and Czeisler, 1995). Therefore, the imposed 2h advance of sleep time during the extension night affected sleep quality, which may have reduced the association between SWE and AD PRS found with baseline and recovery nights.

Polygenic risk for AD is associated with increased subjective daytime sleepiness

We next focused on the non-EEG sleep metrics of our protocol and explored their potential association with AD PRS. Based on the 3 weeks of actigraphy with imposed regular habitual sleep time at home, we computed the probability of transition from rest to activity during the sleep period [kRA; (Lim et al., 2013)]. kRA is a proxy for sleep fragmentation and has been associated with cognitive decline and the risk for developing AD in cognitively normal older adults [mean age 81.6 y (Lim et al., 2013)]. kRA showed a negative association (higher AD PRS is associated with less fragmented sleep) with PRS for AD for two p-value thresholds, $p=5 \times 10^{-8}$ and $p = 10^{-8}$ (**Figure 3.4A; Supplementary Table 3.S5**), but did not reach stringent experiment-wise correction for multiple comparisons ($p < 0.002$); it will not be further discussed.

Two questionnaires assessed habitual subjective sleep quality and daytime sleepiness before the start of the protocol. Subjective sleep quality was not significantly associated with AD PRS. By contrast, subjective daytime sleepiness was significantly associated with PRS for AD ($p < 0.05$; **$\beta \geq 0.11$**) from thresholding at $p < 10^{-4}$ up to a threshold of $p < 1$ and at stringent experiment-wise correction for multiple comparisons at p-value thresholds of $p < 0.05$ and $p < 0.3$ (**$\beta \geq 0.16$**; **Figure 3.4A; Supplementary Table 3.S5**). The association was positive indicating that higher habitual subjective daytime sleepiness was associated with higher AD PRS (**Figure 3.4B**). This shows that the association between AD PRS and sleep need, as assessed by electrophysiology, is not a mere effect of the protocol and is mirrored at the behavioural level during habitual daytime functioning (outside the experimental protocol). Importantly the vast majority of participants had no or mild levels of sleepiness with a minority (N = 28) reporting moderate level of daytime sleepiness; the association with daytime sleepiness is therefore not driven by extreme or clinically relevant sleepiness levels but rather by ordinary variability in healthy young

individuals.

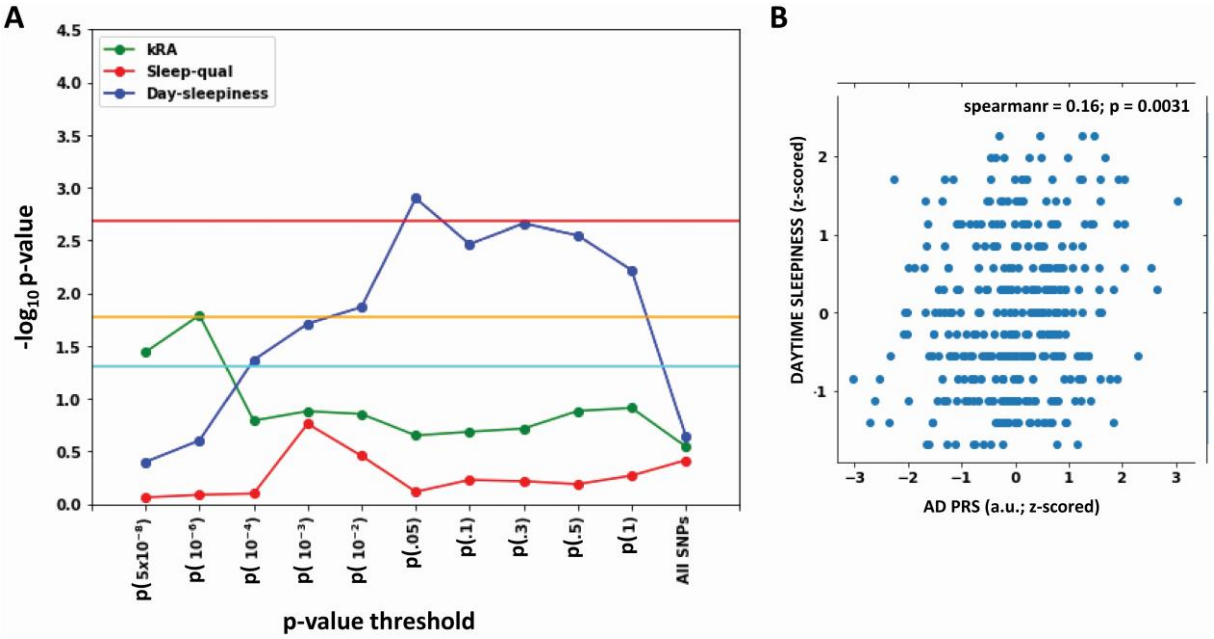

Figure 3.4. Associations between PRS for AD and non-EEG sleep metrics. (A) Statistical outcomes of GLMs with actimetry-assessed sleep fragmentation (kRA; N = 361), subjective sleep quality (Sleep-qual; N = 363), and subjective daytime sleepiness (Day-sleepiness; N = 363) versus AD PRS from conservative ($p < 5 \times 10-8$) to inclusive ($p < 1$) p value thresholds and using all SNPs. GLMs are corrected for age and BMI. The negative log transformation of p values of the associations is presented on the vertical axis. Horizontal lines indicate different p values: light blue = 0.05 (uncorrected); orange= 0.016 (corrected for three sleep metrics); red = 0.002 (experiment-wise correction). (B) The positive association between subjective daytime sleepiness and AD PRS at $p < 0.05$ (N = 363). Linear regression line shown for display purposes only; refer to the main text and Supplementary Table S5 for statistical outputs of GLMs.

Discussion

We provide evidence that genetic liability for AD is related to sleep characteristics and daytime sleepiness in young adults (aged 18 to 31 y), i.e. decades before typical onset age of clinical AD symptoms and at an age at which current AD biomarkers are typically negative. Our sample size is modest for the detection of small effect size associations, and we do not include a replication sample, so the present results should be considered as a proof-of-concept for linking AD liability and sleep in young adults. We emphasize, however, that the unique deep phenotyping of our protocol in hundreds of participants, based on gold standard electrophysiology and comprising different sleep conditions, one the one hand, makes the creation of a replication

sample difficult but, on the other, undoubtedly increased the sensitivity of our analyses so that we could find associations that survived stringent correction for multiple comparisons. In addition, we performed a negative control analysis using a PRS for height variables for which no association with sleep metrics was expected, and found no association. Furthermore the absence of links between PRS for AD and IQ and education suggest that the association between PRS for AD and SWE is more stable across subjects than the link previously isolate between AD and IQ or education (Kunkle et al., 2019). Importantly, our protocol provides links between disease risk and sleep physiology in contrast to coarser phenotyping based on sleep questionnaires or actimetry alone. Furthermore, to increase the genetic uniformity of the sample, we only included Caucasian men within a narrow age range; they were healthy and devoid of any sleep disorders or sleep complaints and their prior sleep-wake history was recorded and stable. In this carefully selected homogenous sample, we show that higher PRS for AD was associated with producing denser or larger slow waves during baseline and recovery night time sleep, potentially with large slow wave sleep rebound following sleep deprivation, and with reporting higher daytime sleepiness.

Larger and more abundant slow waves during habitual sleep in young and healthy individuals can result from an increased sleep need due to insufficient prior sleep (Klerman and Dijk, 2005). This appears unlikely: prior sleep-wake history was stringently controlled for 3 weeks prior to entering the lab, ruling out undue sleep deprivation, sleep restriction or disrupted rhythmicity. Moreover, throughout the protocol, participants followed their own sleep schedule, a regime that should not expose them to important chronic sleep restriction. Finally, SWE during the sleep extension night did not significantly correlate with subjective daytime sleepiness (Spearman's correlation $r = 0.08$, $p = 0.11$), supporting the idea that, when given a longer sleep opportunity, individuals with higher and yet normal daytime sleepiness did not sleep more intensely to recover a putative prior sleep debt. Alternatively, increased slow wave density and/or intensity could reflect a faster build-up of sleep need (Viola et al., 2007). Indeed, sleep homeostasis is thought to result from molecular and cellular changes induced by waking brain function and behaviour (Scammell et al., 2017; Tononi and Cirelli, 2014). Synaptic potentiation and increased synaptic strength resulting from waking experience are reflected in a progressive increased cortical excitability during wakefulness (Huber et al., 2013; J. Q. M. Ly et al., 2016) and an increase in slow wave activity during subsequent sleep (Scammell et al., 2017; Tononi and Cirelli, 2014). Likewise, extracellular glutamate concentration

and glutamatergic receptor density increase with time awake and affect brain function (Dash et al., 2009; Hefti et al., 2013). Here, SWE rebound following sleep loss, i.e. the ratio between baseline and recovery sleep, was only significantly associated with high PRS for AD for one p-value threshold and at uncorrected significance threshold, but was strongly associated with SWE during recovery sleep. We therefore find only partial evidence for this second hypothesis, which will require more investigations.

How are these findings related to AD? The answer to this question remains speculative because the time course of AD processes across lifespan is still poorly understood. In transgenic mice, neuronal activity locally increases the level of Aβ in the interstitial fluid and drives local Aβ aggregation (Bero et al., 2011). The progressive Aβ deposition ultimately disrupts local functional connectivity and increases regional vulnerability to subsequent Aβ deposition (Bero et al., 2012). We might thus hypothesize that individuals with more intense brain activity during wakefulness (and therefore also during sleep) would also be exposed to larger Aβ extracellular levels and a greater risk of developing Aβ deposits. This hypothesis appears unlikely for the following reasons. First, post mortem examinations show that the earliest evidence of Aβ deposits (stage 1 (Thal et al., 2002)) is not observed before 30 y (Braak and Del Tredici, 2015). Second, Aβ oligomers might be released and exert their detrimental effect on brain function at an earlier age. However, in transgenic mice, sleep-wakefulness cycle and diurnal fluctuation in brain extracellular Aβ remain normal until plaque formation (J. H. Roh et al., 2012).

By contrast, given the age range of our population sample, the reported topography of pretangles at this age (Braak and Del Tredici, 2011) and the power of PRS for AD to discriminate AD patients in case-control samples (Escott-Price et al., 2015), higher PRS in our young sample might reflect the influence of incipient Tau aggregation onto sleep regulation through the LC (and other non-thalamic cortically-projecting nuclei, as raphe nuclei) (Braak and Del Tredici, 2011). Tau, an intracellular protein, is also detected in the extracellular space. Over and above a low level constitutive tau secretion (Chai et al., 2012), neuronal activity increases the release of tau in the extracellular space (Yamada et al., 2014), thereby participating in enhancing tau spread and tau pathology in vivo (Schultz et al., 2018). Moreover, early electrophysiological changes indicative of hyperexcitability are observed in intact neurons from transgenic tau mice (Crimins et al., 2012). In the cerebral cortex of tau transgenic mice, glutamatergic and GABAergic neurons are in a hypermetabolic state, characterized by a relative increase in production of glutamate (Nilsen et al.,

2013). By contrast, decreasing tau in epilepsy-prone transgenic mice reduces neuronal hyperexcitability (Holth et al., 2013). These findings would suggest that a strong cerebral activity during wakefulness would result in a higher daily average in perceived sleepiness, a substantial tau release – which lead to the formation of pretangle aggregates - and an enhanced sleep homeostasis processes, as indicated by denser and larger slow waves.

The reasons for the vulnerability of LC to Tau aggregation are not established but might reside in its constant recruitment for essential functions, its energy demanding and ubiquitous brain connections, its high vascularization or its higher susceptibility to oxidative stress (Mather and Harley, 2016a). Although it tantalizing to hypothesize that tau pretangle aggregates are involved in the mechanisms linking slow wave sleep and AD liability, one can also speculate that it is the LC intrinsic characteristics that are related to tau vulnerability (subsequent) that associated with PRS for AD, meaning that the association would not necessarily require the presence of tau to be detected.

On the other hand, in tau transgenic mice, misfolded and hyperphosphorylated tau alters hippocampal synaptic plasticity (Polydoro et al., 2014), eventually induces a loss of hippocampal LTP and causes reduction of synaptic proteins and dendritic spines (Van der Jeugd et al., 2012) (Sydow et al., 2011). These findings would predict a lower sleep need in participants with high AD liability. However, it is possible that these detrimental processes take place later on in the development of the disease or emerge from an interaction between tau and Ab (Oddo et al., 2003) (Fein et al., 2008). Accordingly, in older adults, significant associations, opposite to the current findings, were observed between slow wave sleep and risk for AD based on PET biomarkers (Lucey et al., 2019; Mander et al., 2015): higher Aβ (Mander et al., 2015) or tau NFT (Lucey et al., 2019) burdens were associated with lower sleep slow wave EEG power. Our results suggest therefore that the association between AD risk and sleep homeostasis changes with age: at an early stage, dense and large slow waves would be associated with increased AD risk. Later on, the ability to generate slow waves would play a protective role against AD risk. Deep sleep phenotyping across all ages and/or in long term longitudinal studies will have to test this hypothesis.

We emphasize that the cross sectional nature of our study, precludes any causal interpretation of the association we find between AD and sleep. We further stress that PRS estimation based on all available SNPs may be biased by complex linkage

disequilibrium (LD) between SNPs. Since we find similar p-values when pruning SNPs for LD at other p-value thresholds, we are confident that the likely bias is not the main driver of the effects we report. Furthermore, our sample only include men and cannot therefore be extended to the entire population. Women have been reported to have different sleep characteristics, including the production of more numerous and intense slow waves during sleep (Svetnik et al., 2017). It is also worth mentioning that we cannot isolate in our findings the specific contributions of the circadian timing system, which is the second fundamental mechanism regulating sleep and wakefulness (Dijk and Landolt, 2019). Although we find significant association between AD PRS and baseline/recovery SWE and daytime sleepiness across similar p-value thresholds, more research is also required to determine how many SNPs one has to include, i.e. what SNP selection strategy should be used to best predict AD. Previous studies support that using a lenient p-value thresholds is successful in doing so (Escott-Price et al., 2015; Mormino et al., 2016), thus we are confident that our finding are related to AD liability. Our PRS calculation was stringently controlled for the weight of chromosome 19 (see methods) to avoid excessive contribution from Apolipoprotein E (APOE) genotype, which is the genetic trait most associated with sporadic AD. When comparing APOE ε4 carriers genotype vs. non-carriers, no significant difference in baseline night SWE and daytime sleepiness was observed **(Supplementary Figure 3.S4)**, in line with our findings that a large number of SNPs is required to find an association between SWE and PRS for AD.

The specificity of our findings for a given EEG frequency band and/or for NREM remains to be fully established. As many previous studies on linking sleep and AD risk [e.g. (Lucey et al., 2019; Mander et al., 2015)], we only focussed on a limited set of sleep metrics, and included a single power measure over a given frequency band. Although not the focus of the present paper, we computed SWE, relative SWE (i.e. ratio between SWE and overnight total NREM power), overnight cumulated total power during NREM sleep and overnight cumulated power in the 2 to 6 Hz band during REM sleep of the baseline night in individuals among the higher and lower AD PRS quartile **(Supplementary Figure 3.S5)**. This simple analyses indicates that individuals with 25% highest AD PRS had higher power than individuals with 25% lowest AD PRS for all three absolute measures (t-test; $p \leq 0.01$ but not for relative SWE ($p = 0.14$), suggesting that our findings may not be specific to NREM sleep and SWE. We emphasize, however, that, given our modest sample size, our analyses was not planned to address such question. This first preliminary analysis warrants

future studies with larger sample size ensuring sufficient power when using a larger set of sleep metrics. Since we also find that daytime sleepiness, a wakefulness trait, is associated with PRS for AD, and because of the link between tau protein and cortical excitability (Holth et al., 2013), neuronal activity synchrony during wakefulness should be associated with the risk for developing AD to assess whether isolated links are specific to sleep.

In conclusion, we find that denser and/or more intense sleep slow waves during baseline and recovery sleep and daytime sleepiness are associated with the genetic liability for AD in young and healthy young men. This finding supports that sleep slow wave and sleepiness measures may help early detection of an increased risk for AD and reinforce the idea that sleep may be an efficient intervention target for AD. Similarly to most studies associating PRS to phenotypes of interest [e.g. (Marden et al., 2016; Mormino et al., 2016; Sabuncu et al., 2012; Santoro et al., 2018)], the effects we isolated constitute relatively small effects ($r < 0.2$), however, recalling that sleep must be envisaged within the multifactorial aspect of a complex disease such as AD (Norton et al., 2014).

Chapter 4: Genetic risk for insomnia is associated with objective sleep measures in young and healthy good sleepers

This results section has been published in Neurobiology of disease in 2022. Supplementary materials related to this paper are displayed in **Appendix 3**.

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Abstract

Insomnia disorder (ID) is the second most common neuropsychiatric disorder. Its socioeconomic burden is enormous while diagnosis and treatment are difficult. A novel approach that reveals associations between insomnia genetic propensity and sleep phenotypes in youth may help understand the core of the disease isolated from comorbidities and pave the way for new treatments. We obtained quantitative nocturnal sleep electroencephalogram (EEG) features in 456 participants (18-31y, 49 women). Sleep EEG was recorded during a baseline night following at least 7 days of regular sleep times. We then assessed daytime sleep onset latency in a subsample of N=359 men exposed to manipulations affecting sleep pressure. We sampled saliva or blood for polygenic risk score (PRS) determination. The PRS for ID was computed based on genome-wide common single nucleotide polymorphism assessments. Participants also completed a battery of behavioral and cognitive tests. The analyses revealed that the PRS for ID was negatively associated with cumulated EEG power in the delta (0.5-4Hz) and theta (4-8Hz) bands across rapid eye movement (REM) and non-REM sleep ($p \leq .0026$; $\beta \geq -.13$) controlling for age, sex and BMI. The PRS for ID was also negatively associated with daytime likelihood of falling asleep ($\beta = -.19$, $p = .0009$). Other explorations for associations with non-baseline-nights, cognitive measures, and mood did not yield significant results. These results propose that the need or the ability to fall asleep and to generate slow brain activity during sleep may constitute the core sleep-related risk factors for developing ID.

Introduction

Insomnia disorder (ID) is the most common sleep disorder and the second most common neuropsychiatric disorder(American Psychiatric Association, 2013). ID prevalence estimates range from 6 to 18% in the general population (Ohayon, 2002), but since it is an age-related disorder, it may be as high as 50% in the elderly (Patel et al., 2018). ID is associated with reduced life expectancy (Robbins et al., 2021) and increased risks for diabetes, cardiovascular diseases and psychiatric disorders (Anothaisintawee et al., 2016; Gangwisch et al., 2010), implying that ID constitutes a huge socioeconomic burden – tens of billion for the U.S. alone (Kessler et al., 2011). Yet, ID diagnosis and treatment remain difficult. The diagnosis is exclusively based on self-report. Traditional polysomnographic sleep scoring often fails to find a reduction in total sleep time matching subjective experience (Harvey and Tang, 2012). ID is also much more heterogeneous than initially apprehended (Benjamins et al., 2017) and shows high comorbidity with other diseases, particularly anxiety and depression (Wittchen et al., 2011), which are often preceded by ID. In our aging society, the need for in- depth understanding of ID leading to novel prevention and treatment targets has never been so high.

ID involves a chronic hyperarousal state of increased somatic, cognitive and cortical activation, and negatively impinges on sleep (Riemann et al., 2010). Beyond difficulties falling or remaining asleep, signatures of hyperarousal are even found during sleep, which contains more arousals and fast electroencephalogram (EEG) oscillations. This restless sleep would interfere with the downregulation of emotion and arousal on the short term (Wassing et al., 2019a) and long term (Wassing et al., 2019b). The course of ID is commonly described within the '3P' model which posits predisposing, precipitating and perpetuating factors (Spielman et al., 1987). While several perpetuating factors have been recognized, including poor sleep hygiene and negative beliefs about sleep, much less is known about how predisposing, genetic factors promote the development of insomnia.

Insomnia heritability is substantial, with twin study estimates ranging from 0.28 to 0.59 (Barclay et al., 2021). Genome wide association studies (GWAS) have established that ID is highly polygenic with 202 loci identified using stringent statistical criteria and up to 956 different genes reported in the literature (Jansen et al., 2019). Genetic variants revealed by GWAS explain only a small proportion of phenotypic variation in complex diseases like ID. This phenomenon, known as the missing heritability problem, arises from the infinitesimal contribution of multiple

genetic variations. A common way to aggregate the additive contributions of multiple genetic variants is calculating an individual's polygenic risk score (PRS). The PRS is a weighted sum of the number of risk alleles and provides an estimate of the individual's genetic risk. PRS have been previously used to link the genetic risk for Alzheimer's disease with the cognition (Coors et al., 2022) and with cerebrovascular function (Chandler et al., 2019); schizophrenia with cognitive and neural plasticity (Zhao et al., 2022); other psychiatric disorders with cognition, behaviour and brain imaging (Gui et al., 2022). How a set of risk allele variants contributes to predisposing, precipitating and perpetuating factors of insomnia is however unknown. Revealing how genetic variants affect sleep biology is arguably fundamental to a better understanding of insomnia disorder.

Since individuals devoid of sleep disorders may differ with regards to genetic liability for ID, investigating how the PRS for ID impacts sleep EEG in unaffected young healthy adults may be the best way to obtain clues about the underlying biology of ID. This type of approach would assess whether inherited liability for ID may already manifest in objectively detectable sleep phenotypes in people without disorders, relatively uncontaminated by the variance related to aging, comorbidities and lifetime experiences that would be present in people that have actually developed chronic ID. In a cross-sectional study, we therefore assessed the PRS for ID and sleep EEG in 456 young individuals devoid of any sleep complaints and psychiatric symptoms. We hypothesized that the PRS for ID would be associated with EEG markers of insomnia vulnerability assessed during normal nocturnal sleep (e.g. lower and higher power in slower and faster EEG oscillations, respectively) and under conditions of altered sleep pressure. We then also explored whether the PRS would be associated with subjective metrics of sleep, anxiety and mood, as well as with cognitive consequences of altered sleep pressure.

Material and methods

Standard Protocol Approvals, Registrations, and Patient Consents

All the participants signed an informed consent and experiments were approved by the Ethics Committee of the Faculty of Medicine of the University of Liège.

Participants and protocols

We retrospectively analyzed DNA and in-lab EEG recordings of sleep of 456 young and healthy individuals aged 18 to 31y (22±2.7y; 49 women) collected across 6 different studies conducted at University of Liège, Belgium (**Supplementary Table**

4.S1). All protocols (Gaggioni et al., 2019; J. Q. M. M. Ly et al., 2016; Mascetti et al., 2013; Muto et al., 2021, 2016; Vandewalle et al., 2009) included baseline EEG recordings of night-time sleep at habitual sleep times following at least one week of regular sleep-wake schedules monitored by actigraphy.

Exclusion criteria aimed at constituting a sample of very healthy men devoid of any chronic disease, including sleep disorders and were as follows: body mass index (BMI, kg/m^2) <18 or >29, diabetes, excessive alcohol (>14 units/week) and caffeine (>3 cups/day) consumption, addiction, diagnosed psychiatric disorders, including insomnia, depression and anxiety, shift work during the past year, transmeridian travel in the last 3 months, use of psychoactive drugs, sleep medication. A screening night of sleep under full polysomnography excluded sleep disorders (apnea-hypopnea index ≥15/h; periodic limb movement, ≥15/h; REM sleep behaviour disorder, sleep walking). Participants were requested to refrain from caffeine at least 3 days prior to the study. Anxiety and depression symptoms were assessed with the Beck Anxiety Inventory (BAI) and Beck Depression Inventory (BDI) (Beck et al., 1988; Beck et al., 1988). Sleep quality and sleepiness were assessed with the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) and Epworth sleepiness scale (ESS) (Johns, 1991), respectively.

Some additional analyses were performed in a sub-sample of 359 young healthy men, which is described in details in (Muto et al., 2021).

Sleep EEG metrics

Sleep EEG was recorded using either a V-Amp 16, a QuickAmp-72 (Brain Products GmbH, Germany) or a N7000 (Gaggioni et al., 2019) (Natus, Planegg, Germany) amplifier. EEG montage varied across studies but included at least Fz, Cz, Pz, Oz and mastoid leads, as well as bipolar electrooculogram, electromyogram and electrocardiogram channels. EEG data were digitized at 200 or 500Hz sampling rate. EEG data were re-referenced off-line to the average of both mastoids using Matlab (Mathworks Inc., Sherbom, MA).

Sleep data were scored in 30-s epochs using a validated automatic sleep scoring algorithm (ASEEGA, PHYSIP, Paris, France) (Berthomier et al., 2007). Arousals and artefacts were detected automatically as previously described (Wallant, 2016) and excluded from power spectral density analyses (pwelch matlab function; 4s epochs without artefact or arousal; 2s overlap). Only frontal electrodes were considered because the frontal region is most sensitive to sleep pressure manipulations

(Cajochen et al., 1999) as well as to facilitate interpretation of future large-scale studies using headband EEG, often restricted to frontal electrodes. Averaged power was computed per 30 min bins, adjusting for the proportion of rejected data (containing artefact/arousal), and subsequently aggregated in a sum separately for REM and NREM sleep. Thus we computed slow wave energy (SWE) - cumulated power in the delta frequency band during NREM sleep, an accepted measure of sleep need (Plante et al., 2016), and similar to that we computed the cumulated theta (4-8Hz) power in REM sleep. We then computed the cumulated power over the remaining EEG bands, separately for NREM and REM sleep: alpha (8-12Hz), sigma (12-16Hz) and beta (16-25Hz) bands. The cumulated power score would increase with time spent in REM and NREM sleep, so we included total sleep time (TST) as a common covariate in all analyses, as well we then controlled for REM and NREM sleep duration for REM and NREM sleep power respectively.

Primary analyses focused on six sleep metrics to limit issues of multiple comparisons while spanning the most important aspects of sleep EEG previously associated with ID: 1) sleep onset latency (SOL) and 2) wake after sleep onset (WASO), to assess overall sleep quality and continuity(Perlis et al., 2010); 3) SWE during NREM sleep to assess slow wave generation; 4) cumulated overnight beta power during NREM sleep to quantify high frequency activity (Merica, 1998); 5) the number of arousals during REM sleep to reflect its instability (Riemann et al., 2012); and 6) cumulated theta power during REM sleep to assess its most typical oscillatory activity (Benz et al., 2020).

Quality control of genetic data and imputation

Genotyping was performed at Genomics platform of ULiège GIGA institute using blood samples or buccal swabs and Illumina Infinium BeadChip arrays based on Human Build 37 (GRCh37). The samples were frozen (-20°C) within a few hours following collection and until DNA extraction. Quality control (QC) was performed using PLINK (http://zzz.bwh.harvard.edu/plink/), (Purcell et al., 2007). One participant was excluded from subsequent analyses due to mismatch between actual and imputed sex. No sample presented >10% missing genotypes. We removed SNPs as follows: <95% call rate, <0.01 minor allele frequency (MAF), out of Hardy-Weinberg equilibrium (p-value < 10^{-4} for the Hardy-Weinberg test), on 23rd chromosome, ambiguous SNPs (A-T, T-A, C-G, G-C). For one pair of individuals, the composite pi-hat score was 0.57 suggesting that they are first-degree relatives. We did not exclude these individuals, but removing one subject of this pair did not affect the statistical

significance of any of the tests reported below. As a part of QC, we merged our data with 1000 Genomes Project (1KGP, https://www.internationalgenome.org), and applied principal component analysis on the merged data to verify that our cohort was located in the European cluster **(Supplementary Figure 4.S1A**). We then compared the allele frequencies in our cohort with those of the European subset of the 1KGP **(Supplementary Figure 4.S1B).** We removed SNPs which minor allele frequencies >.2 compared with European subset **(Supplementary Figure 4.S1C-D).**

Imputation was performed using the Sanger imputation server (https://imputation.sanger.ac.uk/) by choosing "Haplotype Reference Consortium (r1.1)" as reference panel and EAGLE2 pre-phasing algorithm. We applied the same QC including allele frequency check with the European cohort of 1KGP. We also excluded SNPs with quality of imputation <.3. After QC, 7300849 SNPs remained for statistical analyses.

Predicting height as part of quality control

As part of quality control, we computed a polygenic score for the height of the participants based on the summary statistics for height which includes 3,290 genome-wide significant loci that explain approximately 25% of the phenotypic variation in height in European ancestry individuals (Yengo et al., 2018). Correlation between polygenic scores based upon these SNPs with actual height in our cohort is 0.4, $p = 1.5*10^{-18}$ **(Supplementary Figure 4.S2),** similar to previously reported (Yengo et al., 2018).

Polygenic risk score computation

The individual polygenic risk score (PRS) in our ID-free sample was calculated as the sum of SNPs associated with ID, weighted by estimated effect sizes of the summary statistics of a large ID case-control GWAS (Jansen et al., 2019). We used the results of the 23andMe sample used in the GWAS (n = 944,477), which is based on case-controls online surveys completion. PRS was computed in PLINK2 using standard approach (Privé et al., 2019): clumping, i.e. markers pruning based on linkage disequilibrium (LD) (r^2=0.2; window size = 1000 Kb), keeping the most significant markers of the GWAS, followed by applying a p-value threshold on GWAS summary statistics to select SNPs of interest (for more details see Supplementary materials).

It has been suggested that the inclusion of a larger number of single nucleotide polymorphisms (SNPs) in the PRS could increase the predictive accuracy and explained variance of diseases (Escott-Price et al., 2015). Yet, the best p-value

threshold for inclusion of a SNP in ID case-control summary statistics to compute PRS is not established. We therefore computed 11 PRSs for insomnia based on increasing p-value thresholds to generate a range of outcomes from including only the SNPs reaching stringent GWAS significance, up to the most liberal threshold (p-value < $5*10^{-8}$, $5*10^{-6}$, $5*10^{-4}$, .001, .01, .05, .1, .3, .5, and 1). We also computed a PRS using all the SNPs, without clumping. **Supplementary Table 4.S2** provides the number of SNPs included in the computation of each PRS.

Statistical analysis

Associations of the PRS with sleep metrics as dependent variables, were evaluated using general linear models (GLM) implemented in Python, with age, BMI, and TST as covariates. Sleep metrics were standardized using a linear Z-transformation. Individual values were considered outliers if > 4SD from the mean and removed from analyses: the number of individuals included in each model is reported below each dependent variable in the **supplementary tables**. For skewed data, generalized linear mixed models (GLMM) were computed in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) to verify significance adjusting for data distribution.

Since the 11 PRS are correlated, we used the singular value decomposition (SVD) of the correlation matrix to estimate the number of independent tests. We ordered the eigenvalues after SVD and calculated the sum of all eigenvalues. We then calculated the minimum number of linear combination of the PRSs that explained 99% of the variance, which resulted in three. Hence, for any of our 6 EEG sleep metric-PRS combination to be statistically significant when taken multiple testing into account, the p-value threshold was set to .05 divided by 3*6, i.e. p = .0028. We computed the minimum detectable effect size given our sample size. According to G*Power 3 (version 3.1.9.4) (Faul et al., 2007), taking into account a power of 0.8, an error rate α of .0028, a sample size of 456 allowed us to detect medium effect sizes r > .21 (2-sided; absolute values; confidence interval: 0.12 – 0.29) within a linear multiple regression framework including 5 predictors.

Data availability

Data are available upon request.

Results

All 11 PRS were normally distributed in our cohort. Statistical analysis controlling for age, sex, BMI and TST first revealed significant negative associations between

the PRS for ID and SWE (p ≤ .045, β ≥ -.09) using the PRS at $p < 10^{-4}$ threshold up to including all SNPs (**Supplementary Table S3, Figure 4.1A**). The association reached stringent experiment-wise corrected significance (i.e. p < .0028) for three p-value thresholds: p = .01, .5, 1 (p ≤.002; β ≥ -.14). Statistical analysis also revealed significant negative associations between the PRS for ID and cumulated theta power during REM sleep (p ≤ .028, β ≥ -.10) using the PRS at $p < 10^{-3}$ threshold up to including all SNPs. Association reached stringent experiment-wise corrected significance for four p-value thresholds: p = .01, .3, .5, 1 (p ≤.0026; β ≥ -.13). The negative sign of the associations (**Figure 4.1B, C**) indicates that people with a higher PRS for ID tend to have less, or less intense, slow waves and theta oscillations, during NREM and REM sleep, respectively. Importantly, associations between the PRS for ID and NREM sleep SWE or REM sleep theta are not driven by the duration of NREM or REM as controlling respectively for NREM and REM sleep duration did not affect statistical outcomes (**Supplementary Table 4.S4**).We further tested for potential associations of PRS for ID with REM and NREM percentage and ratio, and found no significant associations. (**Supplementary Figure 4.S3**). Note that computing the analyses on men only, as well as adding the first two principal components of the genetic data to our models led to the similar statistical outputs (data not shown).

In line with our assumptions, the PRSs for ID computed with *p*-value thresholds of 10^{-3} and 10^{-2}, were also positively associated with the number of arousals during REM sleep (p ≤ .026, β ≥ .09) (**Supplementary Table 4.S3, Figure 4.1A**), but this association did not reach experiment-wise stringent *p*-value correction for multiple comparisons. Contrary to our expectations, the PRSs for ID were not associated with SOL, WASO, and beta power during NREM sleep for any of the PRS p-value thresholds. Interestingly and in line with the literature (Rosinvil et al., 2021), NREM sleep SWE was negatively associated with the age covariate (**Supplementary Table 4.S3**), in spite of the overall young age and limited age range of our sample. NREM sleep SWE was also associated with the sex covariate, with women generating less slow waves than men (**Supplementary Table 4.S3)**. This finding is discrepant with the literature (Rosinvil et al., 2021) and should be taken with caution given the underrepresentation of women in our sample.

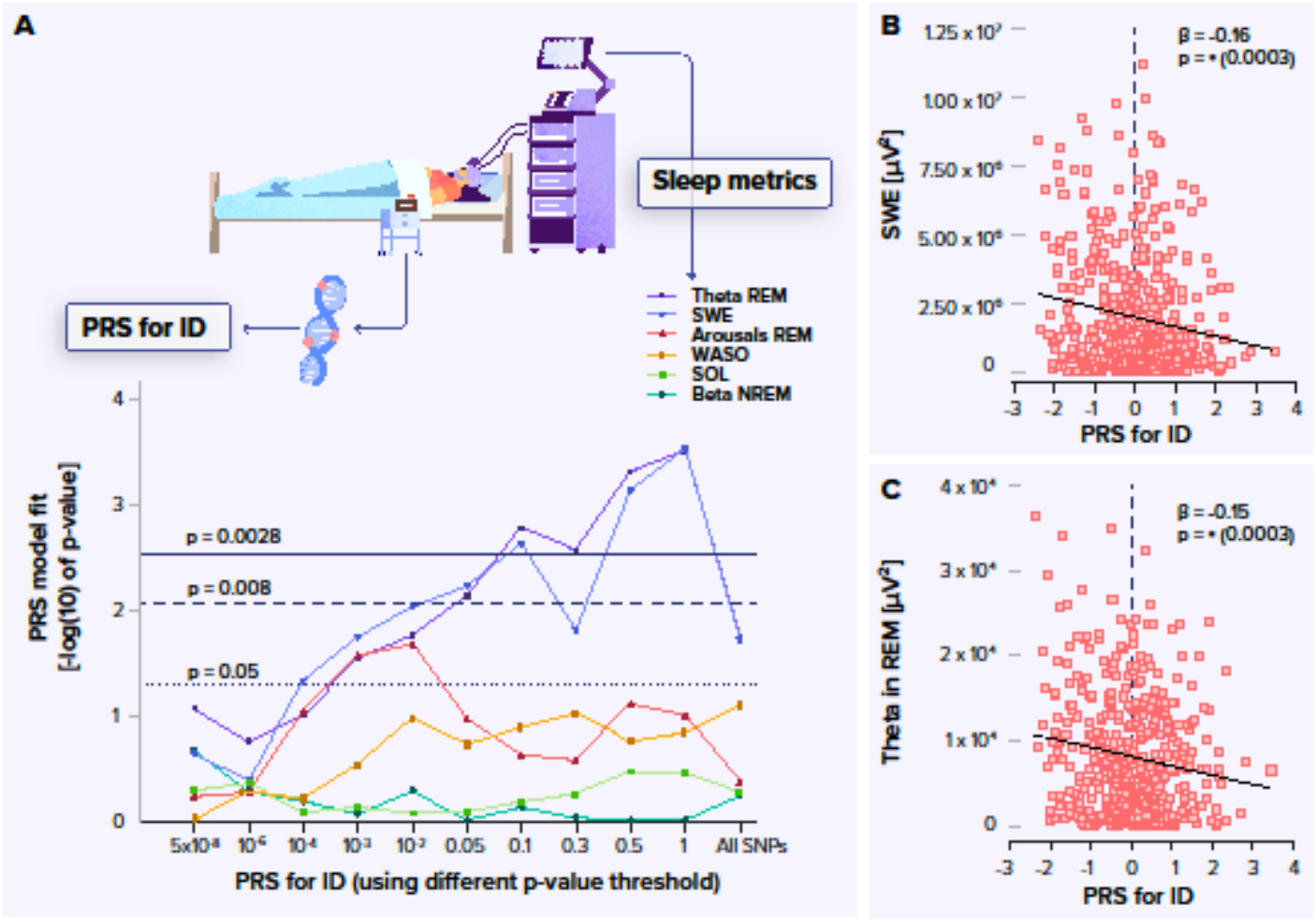

Figure 4.1. Associations between PRS for ID and baseline night sleep metrics.

(A) Statistical outcomes of GLMs with six sleep metrics of interest versus PRS for ID from conservative ($p < 5 \times 10-8$) p value threshold to using all SNPs (N = 456). GLMs are corrected for age, sex, BMI, and TST. Negative log transformation of p values of the associations is presented on the vertical axis. Horizontal lines in A indicate different p values thresholds: dotted = 0.05 (uncorrected); dashed = 0.008 (corrected for six sleep metrics); solid = 0.0028 (experiment-wise correction; see Methods and Materials).

(B) Negative association between SWE (overnight cumulated power in delta - 0.5-4Hz – band) during baseline night and ID PRS including SNPs with p-value threshold = 1 (Spearman r=-0.13, p=0.0075*****).

(C) Negative association between overnight cumulated power in REMS theta (4-8Hz) during baseline night and ID PRS including SNPs with p-value threshold = 1 (Spearman r=-0.11, p=0.014*****).

PRS for ID are expressed in arbitrary units which were z-scored. Spearman's correlations r are reported for completeness and do not substitute the statistical outputs of GLMs which are reported on the graphs and in the Supplementary Table 4.S3. [***** GLM significant association]. **WASO**, wake time after sleep onset; **SOL**, sleep onset latency; **Arousals REM**, number of arousals during REM sleep; **SWE**, slow-wave energy in NREM sleep (0.5–4 Hz); **Beta NREM**, cumulated power in beta (16-25Hz) in NREM sleep; **Theta REM**, cumulated power in theta (4-8Hz) in REM sleep.

Larger effects were found using PRSs with a threshold of p = 1 for SNPs in our primary analyses. Therefore, secondary analyses only used this PRS threshold. As a first secondary analysis, we assessed the specificity of our findings for the EEG frequency bands included in the primary analysis. We tested the associations for other slow rhythms in NREM and REM than in our primary analysis and considered theta power in NREM (rather than NREM SWE) and delta power in REM (rather than REM theta). We found that the PRS for ID was strongly associated with cumulated theta power during NREM sleep ($p = .002$, $\beta = -.13$) and cumulated delta power during REM sleep ($p = .003$, $\beta = -.13$), with p-values below correction threshold for 10 comparisons (i.e. $p < .005$; **Figure 4.2A, B**). It appears therefore that higher PRS for ID is associated with lower power across a lower oscillatory mode ranging from 0.5 to 8Hz both during NREM and REM sleep. The PRS for ID was also negatively associated with cumulated overnight sigma power (12-16Hz) during NREM and REM sleep, but effects did not reach significance following correction for multiple comparisons (**Figure 4.2C, D**) ($p = .01$, $\beta = -.11$). In contrast, the PRS for ID was not associated with alpha and beta power during either NREM or REM sleep ($p > .25$; $\beta < .03$; **Figure 4.2E, F, G, H, Supplementary Table 4.S5).**

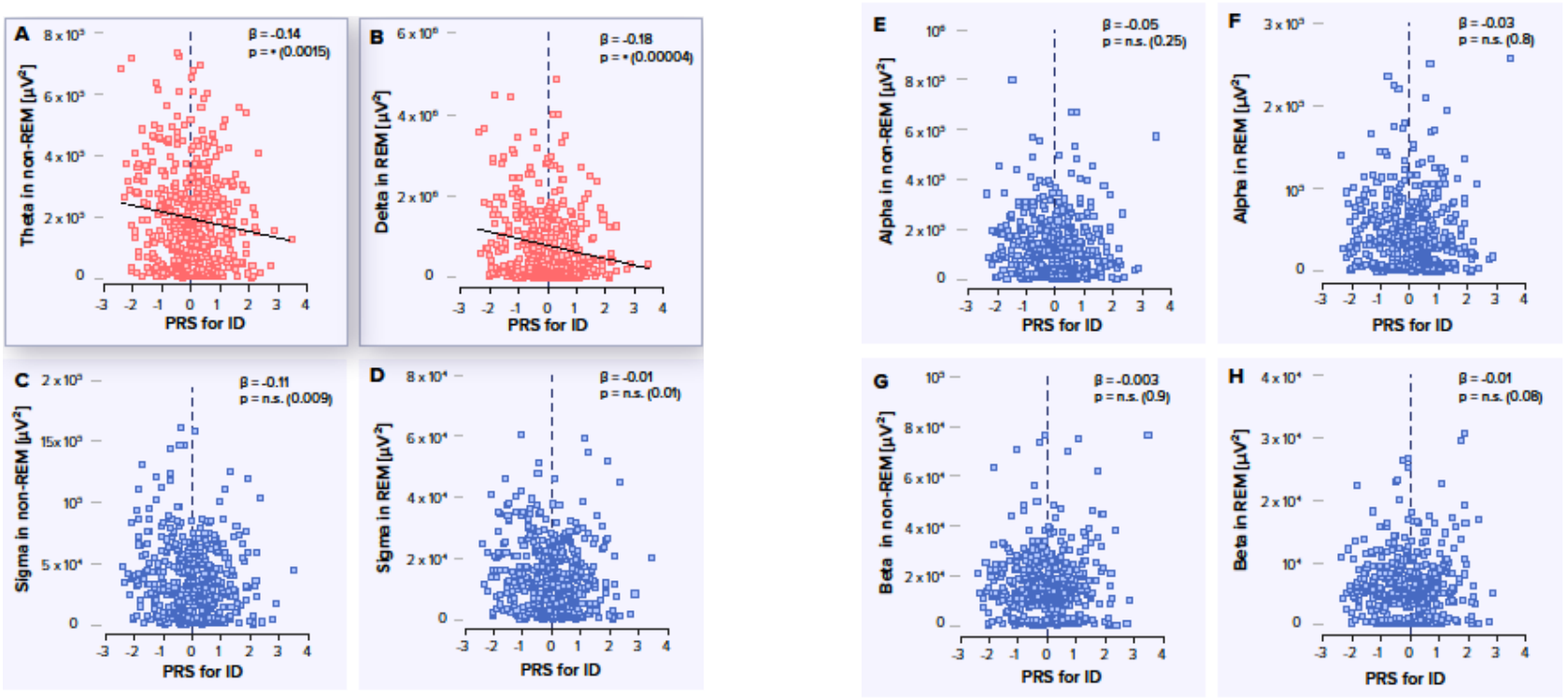

Figure 4.2. Associations between PRS for ID and power in different frequency bands during baseline NREM sleep and REM sleep (N=456)

GLM <u>significant</u> associations between PRS for ID and overnight cumulated power in **(A)** NREM sleep theta (4-8 Hz) (Spearman r=-0.11, p=0.019*****), **(B)** REM sleep delta (Spearman r=-0.13; p=0.0045*****).

GLM <u>non-significant</u> associations between PRS for ID and overnight cumulated power in **(C)** NREM sleep alpha (8-12Hz; Spearman r= -0.069, p=0.14), **(D)** REM sleep alpha (Spearman r=-0.031, p=0.51), **(E)** NREM sleep sigma (12-16Hz; Spearman r=-0.01; p=0.026), **(F)** REM sleep sigma (Spearman r=-0.12; p=0.013), **(G)** NREM sleep beta (16-25Hz; Spearman r=-0.074, p=0.13), and **(H)** REM sleep beta (Spearman r=-0.041, p=0.38). Fitted trend lines are added for visualization purpose, and do not imply that the associations are significant.

PRS for ID (arbitrary units) was computed including SNPs with p-value threshold = 1 and z-scored. Spearman's correlations r are reported for completeness and do not substitute the statistical outputs of GLMs which are reported on the graphs and in the Supplementary Table S6 [***** GLM significant association]

A second ancillary analysis assessed associations between the PRS and non-EEG sleep metrics, including actigraphy-assessed sleep quality, subjective sleep quality and daytime sleepiness, as well as scores on anxiety and depression questionnaires. The PRS for ID was not significantly associated with any of these metrics even at nominal $p < .05$ significance level (**Supplementary Figure 4.S4A-E**).

Subsequent exploratory analyses focussed on a large subsample (N= 359) of our dataset – only composed of men that participated in a 7-day long in-lab protocol including not only baseline sleep but also recordings under three other sleep pressure conditions (sleep extension, recovery following total sleep deprivation, sleep following sleep satiation; as described in (Muto et al., 2021)). The PRS for ID was not significantly associated with either NREM sleep SWE or REM sleep theta assessed during altered sleep pressure conditions, even at nominal $p < .05$ significance level. Importantly however, data of the multiple sleep latency tests(Arand and Bonnet, 2019) that followed baseline sleep in this same 7-day protocol showed that the PRS for ID was negatively associated with the likelihood of falling asleep during these daytime sleep opportunities ($p = .0009$, $\beta = -.19$; **Figure 4.3**). Finally, the PRS for ID was not significantly associated with any of the cognitive measures spanning attentional, memory and executive function domains ($p > .05$) (**Supplementary Table 4.S6**).

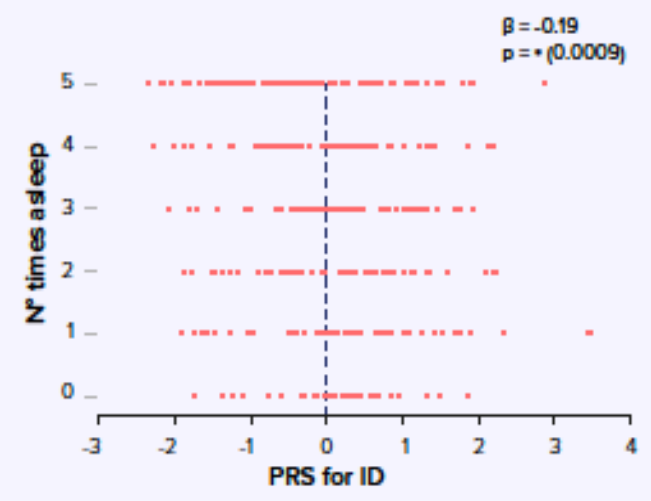

Figure 4.3. Association between PRS for ID and multiple sleep latency test in a subsample (N=359)

GLM significant negative association between PRS for ID and the number of times the individual fell asleep during daytime multiple sleep latency test (Spearman r=-0.21, p=0.00037*****).

PRS for ID (arbitrary units) was computed including SNPs with p-value threshold = 1 and z-scored. Spearman's correlation r is reported for completeness but does not substitute the statistical output of GLM which is reported on the graph and in the main text.

Discussion

In order to reveal sleep markers of insomnia vulnerability, we investigated whether genetic liability for ID is related to sleep metrics in a relatively large sample of polysomnographically assessed young individuals without ID complaints or comorbidities. In line with our hypothesis, we found that higher PRS for ID is associated with poorer sleep as indexed by reduced NREM sleep slow wave energy, i.e. the overnight cumulated power in the EEG delta band during NREM sleep (0.5-4Hz), which reflects sleep intensity. Moreover, the association with slow EEG activity includes both delta and theta range in NREM as well as in REM sleep. The PRS for ID was specifically associated with these frequency bands, and not with cumulated power in the other frequency bands (i.e. 8-25Hz) or sleep metrics not related to EEG power (WASO, SOL, arousal during REM). Overall, our findings indicate that the genetic vulnerability to insomnia involves either a reduced need or reduced ability to generate slow brain activity during sleep. Our study does not indicate, at least not in healthy young adults, a genetic contribution to the short sleep that has also been identified as a risk factor for developing ID (Fernandez-Mendoza et al., 2012).

The hyperarousal that is characteristic of ID is considered to be reflected by delta and theta power deficiency in NREM and REM sleep (Feige et al., 2013; Merica, 1998), which is reminiscent of the associations we found with the PRS for ID. Yet, ID hyperarousal has also been associated with enhanced beta frequency activity during sleep (Perlis et al., 2001), while we did not observe such an association with the PRS for ID. Based on our findings, we hypothesize that the cascade of developing ID primarily involves a genetic predisposition to reduced slow EEG activity during sleep and only secondarily the development of increased beta activity, for example emerging after experiencing precipitating factors (Spielman et al., 1987). The reduced intensity of slower brain activity during NREM and REM sleep may therefore mark a genetic contribution to the vulnerability of developing ID. How this genetic contribution relates to transcription, translation and epigenetic variations cannot be assessed as part of this study but would be of great interest.

It was suggested that hyperarousal may be especially problematic for REM sleep, as stability of REM sleep requires a delicate balance of arousing and de-arousing CNS mechanisms (Riemann et al., 2012). It has been specifically hypothesized that consolidated REM sleep is required to attain a prolonged state of locus coeruleus

silencing and, consequentially, low norepinephrine, which facilitates appropriate memory trace adaptation (Swift et al., 2018). Meanwhile, the high limbic reactivation characteristic of REM sleep suggests replay of emotional activity. Synaptic plasticity taking place during low noradrenalin may thus facilitate favourable adaptation of emotional memory traces. In contrast, restless REM sleep may alter these synaptic processes and even result in sensitisation of emotional memory traces and consequentially hyperarousal (Wassing et al., 2019a, 2019b). The reduced REM theta power we observed in participants with a high PRS for ID may mark the vulnerability to restless REM sleep and its adverse emotional consequences. Indeed, lower REM theta (4-7Hz) power spectral density has been observed in trauma-exposed persons who developed posttraumatic stress disorder compared with those who did not (Gazecki et al., 2018). Theta oscillations during REM sleep involve hippocampus, amygdala and neocortical activity and signal adaptation of emotional memories, including fear memory (Popa et al., 2010).

EEG slow waves, typically found in the delta frequency range during NREM sleep, are also involved in memory consolidation, and moreover provide a readout of homeostatic sleep pressure, which depends in part on the locus coeruleus (González et al., 1996). Slow waves are more prevalent at the beginning of the sleep episode and their intensity depends on the duration of prior wakefulness. The reduced overnight delta activity we found in participants with a high PRS for ID may reflect altered sleep homeostasis.

We also found that it was more difficult for participants with a higher PRS to fall asleep during daytime. This finding argues against the idea that the weaker expression of delta and theta during sleep in participants with a higher PRS for ID would signify an insufficient capacity to dissipate homeostatic sleep pressure, since this would result in a higher daytime sleep propensity. Rather, the findings converge to suggest a relatively specific contribution of the PRS for ID to the expression of slower EEG activity (delta and theta) and the ease of transitioning from wake to sleep. There was no association of the PRS for ID with subjective sleep quality or daytime sleepiness, nor with any of the cognitive tests we administered, which is in line with the fact that cognitive deficits are not that characteristic of ID (Goldman-Mellor et al., 2015).

Limitations of the study design and methodology

We acknowledge that our study bears some limitations. Exclusion criteria were rigorous and not common for large genetic case-control studies. The age range of our sample is

limited to young individuals, while insomnia is more prevalent in older people. We also excluded ID patients during screening and thus we could not estimate the predicted value of our sample when in (Jansen et al., 2019) the PRS explained up to 2.6% of the variance in ID cases. This guarantees, however, that comorbidities related to aging or to ID do not bias our findings. It further provides argument in saying that we may have isolated the core associations between sleep electrophysiology variability and ID genetic risk. A future study could include ID patients and focus on a larger sample. In addition, our sample mainly consisted of men, and the sub-group analyses were performed on men only, so potential sex differences could not be studied here. Furthermore, the reported associations may be not specific to ID and may relate to other psychiatric dimensions such as anxiety or depression, as their genetics highly correlate with ID (Zheng et al., 2022). We stress, however, that we excluded any diagnosed or treated depression or anxiety disorder from our sample of healthy individuals, while the sub-clinical variability in depression and anxiety scales we administered was not related to PRS for ID. Finally, while the method used here for PRS calculation that consists of clumping and p-value thresholding remains widely used, it tends to be superseded by new methods that model LD instead of filtering on it (e.g. LD pred (Vilhjalmsson et al., 2015)). Future studies should consider the LD modeling approach and also more recent GWAS on ID which identified more significant loci (e.g. (Watanabe et al., 2022)).

Conclusions

Using a PRS for ID computed from a large of GWAS (Jansen et al., 2019), we found the genetic risk to involve reduced abilities to express slow EEG activity during nocturnal sleep and to transition to sleep during daytime, which may be common to all ID subtypes. Our use of young and healthy individuals implies that our findings are not biased by comorbidities that are common in later life ID. Although current GWAS and therefore for PRSs derived from them only explain a limited part of the phenotypic variance of complex diseases (Dudbridge, 2013), our findings show that reduced abilities to express slow EEG activity during sleep and to transition to sleep may be at the core of ID and predisposition for ID.

Chapter 5: In vivo locus coeruleus activity while awake is associated with REM sleep quality in healthy older individuals

This results section has been submitted to *eLife* in February 2023. Supplementary materials related to this manuscript are displayed in **Appendix 4**.

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Abstract

The locus coeruleus (LC) is the primary source of norepinephrine (NE) in the brain, and the LC-NE system is involved in regulating arousal and sleep. It plays key roles in the transition between sleep and wakefulness, and between slow wave sleep (SWS) and rapid eye movement sleep (REMS). However, it is not clear whether the LC activity during the day predicts sleep quality and sleep properties during the night, and how this varies as a function of age. Here, we used 7 Tesla functional Magnetic Resonance Imaging (7T fMRI), sleep electroencephalography (EEG) and a sleep questionnaire to test whether the LC activity during wakefulness was associated with sleep quality in 52 healthy younger (N=33; ~22y; 28 women) and older (N=19; ~61y; 14 women) individuals. We find that, in older, but not in younger participants, higher LC activity, as probed during an auditory mismatch negativity task, is associated with worse subjective sleep quality and with lower power over the EEG theta band during REMS (4-8Hz), which are two sleep parameters significantly correlated in our sample of older individuals. The results remain robust even when accounting for the age-related changes in the integrity of the LC. These findings suggest that the activity of the LC may contribute to the perception of the sleep quality and to an essential oscillatory mode of REMS, and that the LC may be an important target in the treatment of sleep disorders and age-related diseases.

Introduction

Sleep is essential to health. Insufficient or poor sleep impacts cognitive, attentional and learning abilities at all ages (Ferrie et al., 2011; Lo et al., 2016; Zavecz et al., 2020), while in the long run, it also increases the risk of developing diabetes (Anothaisintawee et al., 2016), cardiovascular diseases (Bjorvatn et al., 2007; Gangwisch et al., 2010), mood disorders (Harvey, 2011; Johnson et al., 2006) and neurodegeneration (Van Egroo et al., 2019c). Sleep quality declines over the adult lifespan, with an elevated rate of sleep complaints and sleep disorders in aging individuals (Mander et al., 2017). These could arguably contribute to the higher prevalence of psychiatric and neurological diseases reported at older ages (Gulia and Kumar, 2018). Here, we posit that the link between sleep quality and aging arises, at least partly, from the locus coeruleus (LC), a small nucleus in the brainstem.

The LC constitutes the primary source of norepinephrine (NE) in the central nervous system and sends ubiquitous monosynaptic projections to almost all brain areas. The LC-NE system plays a primary role in many aspects of brain functions, including the maintenance of wakefulness (Poe et al., 2020), sleep onset, and the alternation between slow wave sleep (SWS) and rapid eye movement sleep (REMS) (Osorio-Forero et al., 2022; Van Egroo et al., 2022). The integrity of the LC is progressively altered over adulthood. The LC contrast measured using magnetic resonance imaging (MRI) and considered to reflect the neuronal density of the LC, increases up to about 60 years of age and then declines afterwards (Liu et al., 2019; Shibata et al., 2006). The LC is also one of the first brain sites to show, in otherwise healthy individuals, pretangle tau material that is later co-localized with insoluble tau tangles, and synuclein inclusions, which are the hallmarks of the neuropathology of Alzheimer's and Parkinson's diseases (AD, PD), respectively (Braak et al., 2011b; Heiko Braak and Del Tredici, 2011). Importantly, degeneration of the LC neurons contributes to the pathophysiology of REMS behavioral disorder, a preclinical PD condition (Boeve et al., 2007b). It is therefore plausible that the age-related changes in LC integrity affect its functions and contribute, in turn, to the age-related alterations in sleep quality.

Despite the strong link between the LC and sleep, most of the research on this topic was conducted on animal models. There is conflicting evidence in early studies on the consequences of the LC lesions on sleep-wake states, and more recent research have

demonstrated that the inhibition of the LC reduces time spent in wakefulness and its activation leads to sleep-to-wake transitions (Carter et al., 2010). Furthermore, the duration of REMS and the probability REMS – non-REMS (NREMS) transitions did not directly depend on the LC inhibition / stimulation, which implies a modulatory involvement of the LC to REMS rather than a direct contribution to its genesis. Importantly, in human research, poor structural integrity of the LC (assessed with dedicated-MRI-derived LC contrast) was recently linked to a higher number of nocturnal awakenings in older cognitively unimpaired individuals, especially in the presence of AD biomarkers (Van Egroo et al., 2021a). Translation of animal findings to human beings may not be straightforward (Poe et al., 2020), and to date, there is no report of an *in vivo* assessment of the LC functioning in relation to sleep characteristics. This is likely due to the deep position and the small size of the LC - ~15 mm long, ~2.5 mm diameter, ~50.000 neurons (Keren et al., 2009). Part of these limitations are being lifted by the advent of ultra-high field 7 Tesla (7T) MRI which provides a higher signal-to-noise ratio and a higher resolution than most commonly used 3T MRI.

The difficulty of imaging the LC also arises from the fact that the tonic activity of the LC, which is state dependent, is reduced during SWS compared to wakefulness and (almost) absent during REMS, while its highest during wakefulness fluctuating with the level of attentiveness (Aston-Jones and Bloom, 1981; Aston-Jones et al., 2007). Asides from their tonic mode of activity, the LC neurons can also function following a phasic mode (Devilbiss, 2019). Phasic bursts happen in response to salient stimuli, and performance to an attentional task follows inverted-U shaped curve depending on the interplay between phasic and tonic discharge activity (Devilbiss, 2019). One could therefore argue that variability in LC function as captured in an attentional task reflects the variability in the processes modulated by the LC, including sleep.

Here, we tested whether the LC activity probed during wakefulness, is associated with the quality of sleep in healthy younger and older late middle-aged individuals, respectively aged 18 to 30 and 50 to 70 y. Participants' brain activity was recorded in a 7T MRI scanner while they completed a mismatch negativity attentional task, which mimics novelty and salience detection and is known to elicit LC activity (Murphy et al., 2014). Subjective sleep quality was assessed by a questionnaire, together with objective sleep measures as extracted from electroencephalogram (EEG) recordings during a

nocturnal sleep session. We hypothesized that higher activity of the LC during wakefulness would be associated with worse subjective and objective sleep quality.

Results

Fifty-two healthy participants with no history of cognitive and sleep disorders completed the study, including 33 young adults (22.3 ± 3.2 y; 28 women) and 19 late middle-aged individuals (61.05 ± 5.3 y; 14 women) (**Table 5.1**). They first completed a structural 7T MRI session, which served as habituation to the MR environment, and allowed reconstruct a high-resolution whole-brain image as well as a dedicated LC specific image (**Figure 5.1A**). The latter was used to create individual LC-masks in each participant's brain space that were averaged into a group-wise LC-mask in a standardized brain space (see methods). Participants were requested to sleep regularly prior to completing a fMRI session (see methods) in the morning, 2 to 3h after wake-up time, during which they performed an auditory oddball task (**Figure 5.1C**) (Murphy et al., 2014). Participants further provided a subjective evaluation of their habitual sleep quality using a validated questionnaire: the Pittsburgh Sleep Quality Index (PSQI, (Buysse et al., 1989)). Their habitual baseline sleep was recorded in-lab under EEG to extract our main objective sleep features of interest spanning some of the most canonical characteristics of sleep (**Figure 5.1B**): sleep onset latency, related to sleep initiation; sleep efficiency (ratio between sleep time and time in bed), to assess overall sleep quality and continuity; REMS percentage, to reflect the global architecture of sleep; slow wave energy (SWE) during SWS (cumulated overnight 0.5-4Hz EEG power), an accepted marker of sleep need related to the intensity of SWS (Plante et al., 2016); and the cumulated overnight power over the theta band of the EEG (4-8 Hz) during REMS, associated with REMS intensity over its most typical oscillatory activity.

Table 5.1: Characteristics *of the study sample.*

	Late middle-aged (n = 19)				***Young (n = 33)***				
	Mean	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**p-value**
Age	61	5.3	53	70	22	2.8	18	29	0.000
BMI (kg/m^2)	24.9	3.52	19.4	30.9	21.96	3.11	17.2	28.4	0.005
Education (years)	14.58	2.63	9	19	14.5	2.28	12	20	0.81
BDI	5.58	4.05	0	14	6.73	4.2	0	20	0.26
BAI	3.16	3.13	0	9	3.94	2.9	0	11	0.33
ESS	5.52	3.82	0	13	7	3.86	0	14	0.15
PSQI	3.95	2.22	0	8	4.61	1.97	1	9	0.24
Sex (F - M)	14 F – 5 M				28 F – 5 M				0.15

The education level is expressed in the number of years of formal schooling. BMI stands for Body Mass Index. BDI score, BAI score, ESS score and PSQI respectively stand for Beck Depression Inventory score (A. T. Beck et al., 1988), Beck Anxiety Inventory score (Beck et al., 1988), Epworth Sleepiness Scale score (Johns, 1991) and Pittsburgh Sleep Quality Index (Buysse et al., 1989). F: Female and M: Male.

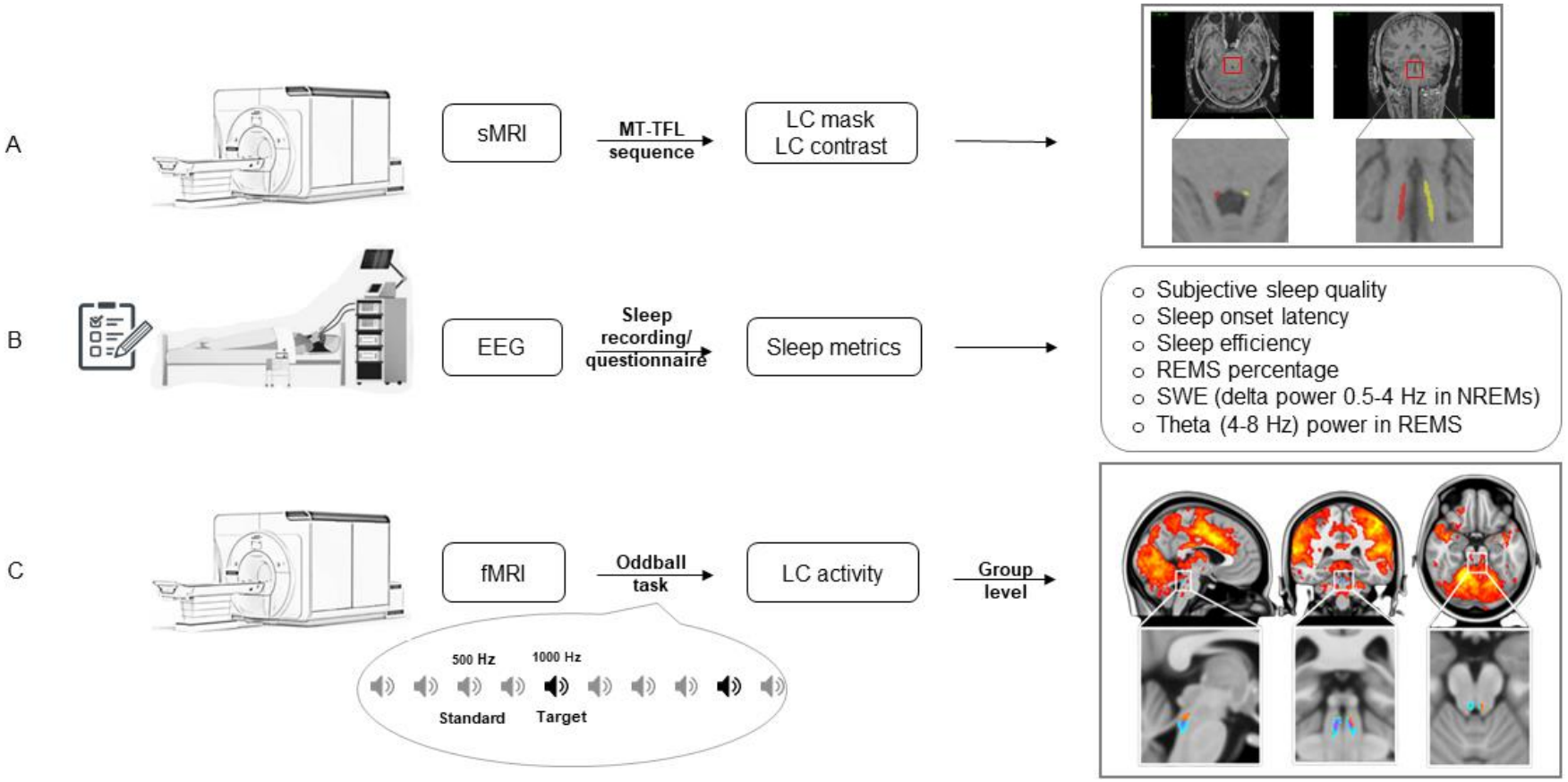

Figure 5.1. Overview of the study protocol.

(A) The volunteers completed a structural 7T MRI (sMRI) session including a sequence for the segmentation of the LC. The latter was used to create individual LC-mask in each participant's brain space as shown in a representative subject (red: left LC; yellow: right LC) and to compute the LC contrast, reflecting the structural integrity of the LC.

(B) Participants' habitual baseline sleep data was recorded overnight in-lab under EEG before the fMRI session to extract our main objective sleep features of interest. They further provided a subjective evaluation of their habitual sleep quality using a validated questionnaire.

(C) After the baseline night, participants underwent a fMRI session during which they completed an auditory oddball task. Brain responses to the deviant tones are displayed as in (Berger et al., 2023) over the group average brain structural image [top row; $p<.001$ uncorrected, right color legend refers to t-values between 3.26 – red - and 8 – yellow] (top) and only over the group-wise of the LC built based on individual LC masks (bottom row; $p<.05$ FWE corrected).

As already reported in this sample (Berger et al., 2023), we found significant activations within the bilateral (though more left lateralized) rostral part of the LC for the detection of the target sound (**Figure 5.1C**; p <.05, FWE corrected for multiple comparisons over the group-wise LC-mask), supporting a robust LC activation during the oddball task. Individual estimates of LC activity were then extracted within the entire individual LC masks in the participant brain space, for higher accuracy, and contrasted against the sleep metrics of interest.

Our first statistical test asked whether habitual subjective sleep quality was related to the activity of the LC. A generalized linear mixed model (GLMM) with subjective sleep quality as the dependent variable found a main effect of LC activity (**p = 0.017**) and age group (**p = 0.046**), as well as a significant LC-activity-by-age-group interaction (**p = 0.006**), whilst controlling for sex and BMI (**Table 5.2**). Post-hoc tests revealed that higher LC activity was associated with worse subjective sleep quality in the older (t = 2.81, **p = 0.007**), but not in the younger group (t = -0.77, P = 0.45) (**Figure 5.2A**).

Table 5.2: **Statistical outcomes of GLMMs with the six baseline night sleep metrics of interest vs. the LC activity**

Sleep metric	***LC activity***	***Age group***	***LC activity *group***	***Sex***	***BMI***	***TST***
PSQI	**F(1,46) = 6.12 P = 0.017**	**F(1,46) = 4.2 P = 0.046**	**F(1,46) = 8.45 P = 0.006*** **	F(1,46) = 0.18 P = 0.7	F(1,46) = 0.71 P = 0.4	
Theta in REMS	F(1,45) = 2.91 P = 0.09	F(1,45) = 0.52 P = 0.5	**F(1,45) = 4.61 P = 0.037#**	F(1,45) = 0 P = 0.96	F(1,45) = 3.65 P = 0.6	F(1,45) = 0.78 P = 0.4
SWE	F(1,45) = 0.18 P = 0.7	F(1,45) = 0.76 P = 0.4	F(1,45) = 0.37 P = 0.6	F(1,45) = 0.46 P = 0.5	F(1,45) = 0.41 P = 0.5	F(1,45) = 0 P = 0.96
SOL	F(1,46) = 1.26 P = 0.3	F(1,46) = 2.46 P = 0.1	F(1,46) = 0.37 P = 0.4	F(1,46) = 0 P = 0.97	F(1,46) = 0.05 P = 0.8	
SEff	F(1,46) = 1.26 P = 0.18	**F(1,46) = 9.81 P = 0.003**	F(1,46) = 3.19 P = 0.08	F(1,46) = 0.01 P = 0.92	F(1,46) = 2.64 P = 0.11	
REMS percentage	F(1,46) = 0.65 P = 0.42	**F(1,46) = 9.16 P = 0.004**	F(1,46) = 1.39 P = 0.2	F(1,46) = 0.2 P = 0.7	F(1,46) = 0.39 P = 0.5	

The GLMs included sex, age, BMI and TST for the power metrics. **PSQI**: Pittsburgh Sleep Quality Index (Buysse et al., 1989), **Theta in REMS**: accumulated theta (4-8Hz) power in REM sleep, **SWE**: slow wave energy (overnight cumulated delta - 0.5-4Hz – power) in NREM sleep, **SOL**: sleep onset latency; **SEff**: sleep efficiency, **REMS percentage**: percentage of REM sleep relative to TST. The number of asterisks close to the p-value indicate corrected significance: '***' means that the association experiment-wise significant, i.e. p-value <0.016 corresponding to the FDR correction threshold; '#' p-value = 0.016 when remove 2 putative outliers [≥ 3 SD & < 4.5 SD for LC activity and REMS theta respectively].

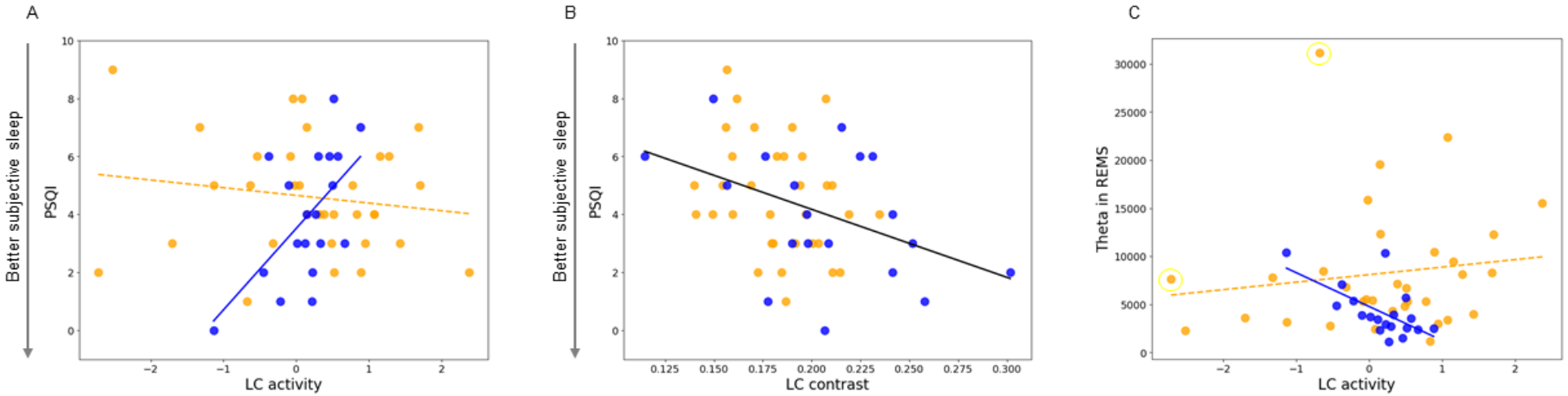

Figure 5.2. Associations between the LC and sleep metrics.

(A) Association between habitual subjective sleep quality, as indexed by PSQI, and the activity of the LC. The GLMM yielded a significant age group by LC activity interaction and post-hoc analyses led to a significant association for the older but not the young group (cf. Table 2).

(B) Association between habitual subjective sleep quality, as indexed by PSQI, and the LC contrast. The GLMM yielded a significant main effect of LC activity (cf. Table S1).

(C) Association between the REMS theta power (cumulated overnight 4-8 Hz EEG power) and the LC activity with age-group interaction. The GLMM yielded a significant age group by LC activity interaction and post-hoc tests led to a significant association for the older but not the young group (cf. Table 2). The 2 highlighted dots correspond to two putative outliers [≥ 3 SD & < 4.5 SD for LC activity and REMS theta respectively] and we note that the p-value of the LC activity by age group interaction goes down to p = 0.12 when removed from the analyses.

Orange dots represent younger individuals (18-30 y, N = 33) while the blue dots represent older individuals (50-70 y, N = 19). Simple regression lines are used for a visual display and do not substitute the GLMM outputs. The black line represents the regression irrespective of age groups (young + old, N=52). Solid and dashed regression lines are used for significant and non-significant outputs of the GLMM, respectively.

The LC activity was computed as a mean of the activity estimates (betas) associated with the appearance of the target sounds in the bilateral LC mask of each subject, within the subject space. Displays are similar when using the left and right LC separately. Subjective sleep quality was estimated using the Pittsburgh Sleep Quality Index (PSQI) (Buysse, et al. 1989) where a higher score is indicative of some sleep difficulties.

We computed the same GLMM using the mean activity of the left and right LC separately to assess whether there is a lateralized association. We obtained similar statistical outputs, though more prominently when focusing on the left LC (main effect of LC activity – left: F= 5.1, **p= 0.03** – right: F= 2.46, p= 0.12; left and right LC-activity-by-age-group interactions: F> 5, **p≤ 0.03**). Both with the left and the right LC activity, post-hoc tests yielded a significant correlation in the older (t > 2, **p≤ 0.04**), but not in the younger group (-0.9 < t < 0, p > 0.39). Furthermore, since we previously reported in our sample a significant age group difference in LC integrity – as indexed by its contrast (see methods and (Berger et al., 2023)) - we added LC contrast as a covariate in the GLMM which yielded the same main effects of LC activity and LC-activity-by-age-group interaction (**Table 5.S1**). Interestingly, the GLMM yielded a significant main effect of LC contrast (F = 5.34; **p=0.025**), with higher LC contrast associated to better sleep quality (**Figure 5.2B**) and no significant interaction between LC contrast and age group (p > 0.9)

We then considered the objective measures of sleep extracted from the EEG. A GLMM with the REMS theta power as the dependent variable found no main effect of the LC activity nor of age while it yielded a significant LC-activity-by-age-group interaction (**p = 0.037**), controlling for sex, BMI and total sleep time (TST) (**Table 5.2**). Post-hoc tests revealed that higher LC activity was associated with lower REMS theta power in the older (t = -2.02, **p= 0.049**), but not in the younger group (t= 0.81, p= 0.42) (**Figure 5.2C**). In addition, removing two putative outliers [≥ 3 SD & < 4.5 SD for LC activity and REMS theta respectively], the LC-activity-by-age-group interaction becomes even more robust (**p = 0.012; Figure 5.2C**). We then computed the same GLMM for mean activity of the left and right LC separately. The REMS theta power was significantly related to the activity of the left LC as a main effect (F= 4.49, **p= 0.04**) and there was an interaction with age (F = 5.33, **p = 0.026**), while no similar association was detected when using the activity of the right LC (F < 1.85, p > 0.15). Similar to the bilateral activity of the LC, post-hoc tests indicated that a higher activity of the left LC was related to lower REMS theta power in the older (t = -2.33, **p = 0.024**), but not in the younger group (t = 0.38, p = 0.7), while no similar association was found when focusing on the right LC (-1 < t < 1, p ≥ 0.3). As for subjective sleep quality, we added LC contrast to the GLMM which yielded the same LC-activity-by-age-group interaction, while no main effect of LC-contrast was detected (F = 0.05; p = 0.8) (**Table 5.S1**). Lastly, if we control for REMS duration rather

than for TST in the GLMM, statistical outputs lead to similar statistical tendencies (main effect of bilateral LC activity: p = 0.067; LC activity by group interaction: p = 0.055).

Importantly, none of the other sleep EEG metrics of interest were significantly associated with the activity of the LC (bilaterally or left and right separately) (**Table 5.2**), suggesting that the association was specific to the subjective sleep quality and REMS theta power. Given the close association between perceived sleep quality and REMS (Della Monica et al., 2018), we tested whether subjective sleep quality was correlated with the theta power in REMS in the older group, and we found a significant correlation with a large effect size (r = -0.54, **p = 0.016**) (**Figure 5.3A**), while the correlation was not significant in younger group and across the entire group (r = -0.26, p = 0.14; r = -0.22, p = 0.12). We further computed a mediation analysis that was purely exploratory given the size of the older subsample, to test whether the theta power in REMS mediated the association between the activity of the LC and subjective sleep quality in older individuals. The analyses yielded no statistical support for mediation (**Figure 5.3B**). While the direct link between LC activity and subjective sleep quality was significant (65.4 ± 32.8% of total effect; p = 0.046), the alternative indirect link was not significant (29.3 ± 41.4% of total effect; p = 0.48).

To gain further insight into the association between LC activity and REMS, we explored the association with additional REMS metrics, including the number of arousals during REMS and REMS episode duration. GLMMs with either of these metrics as the dependent variable did not lead to a significant main effect of LC activity ($F \leq 2.05$; $p \geq 0.16$) nor LC-activity-by-age-group interaction ($F \leq 3.03$; $p \geq 0.08$).

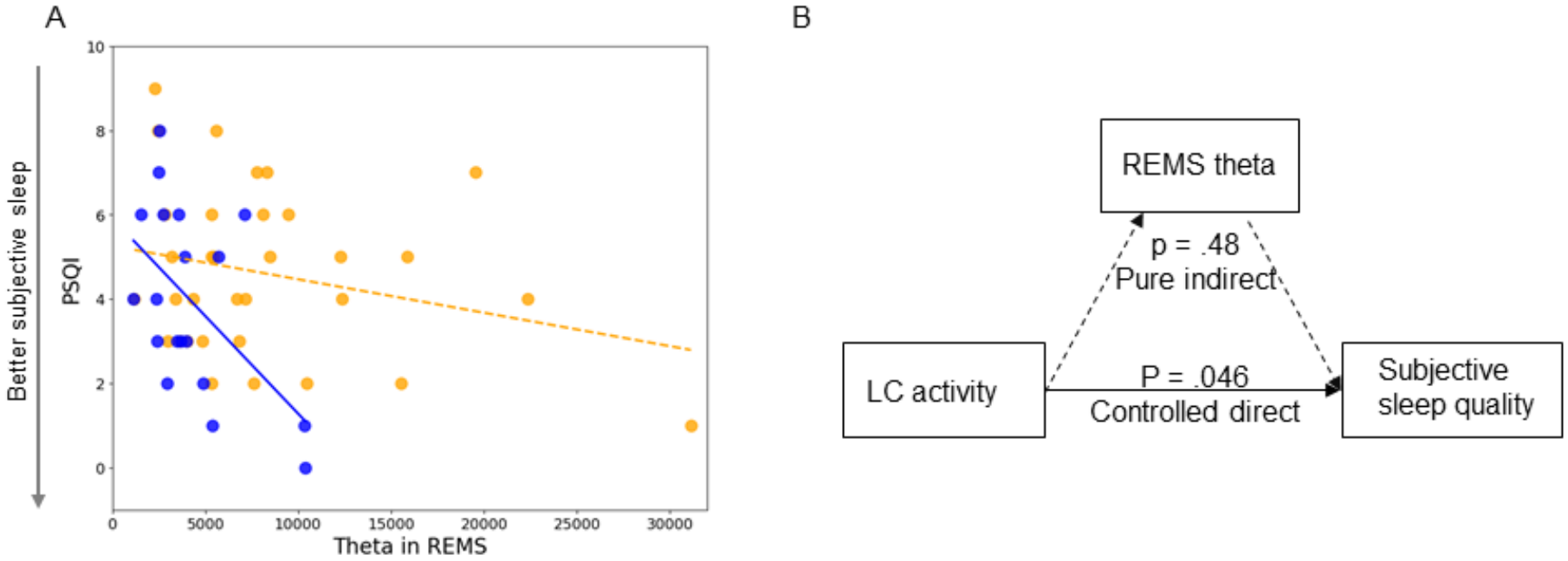

Figure 5.3. Associations between subjective sleep quality and REMS theta power.

(A) Pearson's correlation between habitual subjective sleep quality, as indexed by PSQI, and REMS theta power in the older group (N=19) (r = -0.54, P = 0.016). The orange dots represent individuals of the younger group (18-30 y, N = 33), and the blue dots represent individuals of the older group (50-70 y, N = 19). Solid and dashed regression lines are used for significant and non-significant Pearson's correlations, respectively.

(B) Mediation analyses in older individuals did not provide support for a mediation of the effect between the activity of the LC and subjective sleep quality by REMS theta power.

The LC activity was computed as a mean of the activity estimates (betas) associated with the appearance of the target sounds in the bilateral LC mask of each subject, within the subject space. The LC contrast was computed as the mean contrast of the bilateral LC. Subjective sleep quality was estimated using the Pittsburgh Sleep Quality Index (PSQI) (Buysse, et al. 1989)

Discussion

The LC is arguably one of the most important sleep-wake centers in the brain, and a growing number of animal and human studies have provided evidence supporting its role in regulating sleep and wakefulness, but that the precise mechanisms remain unknown. We provide evidence that, in contrast to young adults (18-30y), higher LC activity during wakefulness is associated with worse subjective sleep quality in late middle-aged individuals aged 50 to 70 y, cognitively unimpaired and devoid of sleep disorders. In addition, we show that higher LC activity during wakefulness is related to lower intensity of REMS in the older but not in the younger subsample. We further find that higher integrity of the LC, as indexed by the LC contrast, is associated with the better habitual subjective sleep quality across the entire sample.

In vivo recording of the activity of the LC during sleep is difficult. On top of its small size and deep position, the tonic activity of the LC is reduced and absent, respectively, during SWS and REMS, while generating sleep, and particularly REMS, in an MRI apparatus is not easy. However, fMRI is sensitive to individual variability in LC activity, whether during sleep or wakefulness, and one could argue that if the LC does not respond or activate as it should during a task, it might also indicate disrupted functional integrity and could be linked to impaired LC-modulated processes, such as sleep. In this first attempt to link LC function to the perceived quality of sleep and its electrophysiology *in vivo* in humans, we posit that the levels of activity of the LC during wakefulness and during sleep are directly related to one another. We decided to use a task known to induce a robust response of the LC during wakefulness (Murphy et al., 2014) and link it with sleep features of interest.

With this in mind, the associations we find between LC activity, subjective sleep quality, and REMS intensity could arise from a negative impact of a higher activity of the LC during sleep (i.e. the higher activity we detected during wakefulness would “bleed” into sleep and correspond to a higher activity of the LC during sleep). In contrast to SWS, REMS quality, as indexed through the number of awakenings during REMS and the duration of REMS, constitutes a predictor of perceived sleep quality (Della Monica et al., 2018). One could therefore hypothesize that it is through the disturbance of REMS that the LC activity is associated with the perception of sleep quality. Although the significant correlation we find between the REMS theta power and the subjective sleep quality brings some support to this assumption, the

mediation statistical analysis which we computed does not corroborate it. Future research should re-assess this mediation in a larger sample of old individuals.

Sleep quality begins to decline at age 40, and sleep complaints rise as well as adults get older (Carrier et al., 2011; Schmidt et al., 2012). Many of the sleep alterations observed in aging and pathologies may arise from subcortical nuclei including the LC (Van Egroo et al., 2022). We find that the association between the LC activity and sleep changes with age: higher LC activity is associated with poorer sleep quality and less intense REMS in middle-aged individuals and not in younger ones. Our findings support the idea that LC activity during sleep could shape part of the large inter-individual variability found in sleep disruptions, particularly starting at an age when sleep becomes more fragile, contributing therefore to age-related sleep complaints.

The LC modulates cortical activity through a tonic or phasic neuronal firing. A trade-off between these two modes allows for maximising the reward and the utility of incoming stimuli (Aston-Jones and Cohen, 2005). Phasic bursts of LC-NE neurons are elicited when confronted with novel or salient stimuli such as in the oddball task we administered (Aston-Jones and Bloom, 1981; Krebs et al., 2018; Rajkowski et al., 1994). Yet, our findings likely depend on the combination of tonic and phasic activity of the LC. Early LC damage has been suggested to result in a state of persistent high tonic LC activity that may disrupt task-related phasic activity (Elman et al., 2017). In addition, the temporal resolution of our fMRI data acquisition is relatively low compared to the burst of action potentials of LC (i.e., one volume was acquired in 2.34s). It is therefore hard to disentangle tonic and phasic contribution to our findings.

Interestingly, the LC, which is functionally connected to the salience network during wakefulness (Song et al., 2017), presents an abnormal functional connectivity pattern in patients with insomnia disorder (Gong et al., 2021), which is the second most prevalent psychiatric disorder (Van Someren, 2021). This abnormal connectivity could contribute to the general state of hyperarousal characterizing insomnia disorder during both wakefulness and sleep to impede restful REMS (Van Someren, 2021). This assumption may underlie the association we find between LC activity and theta power during REMS. REMS theta activity is lower in patients with post-traumatic stress disorder, a condition often associated with insomnia, and higher REMS theta activity predicts a lower chance of re-experiencing symptoms following a stressful event (Sopp et al., 2019). Theta oscillations during REMS are considered to be essential for the hippocampus-dependent memory consolidation during sleep (Boyce et al., 2016), and they serve as the homeostatic control of REMS (Bjorness et al.,

2018). Theta oscillations of REMS take place during a unique behavioral state when the LC is quasi-silenced, providing the conditions for the neuronal potentiation and de-potentiation required for a rewiring of the memory schemas depending on the hippocampus (Poe, 2017; Van Someren, 2021). Consequently, the negative association between LC activity and REMS theta power we find could reflect a relatively more restless REMS when the LC is insufficiently silenced with potential disruption in synaptic plasticity and memory consolidation (Swift et al., 2018). REMS would therefore be maladaptive to the dissolving of distress leading to a higher level of general anxiety. Here, we probed LC activity using an oddball task known to recruit the salience network (Berger et al., 2023) and we used a sample of individuals devoid of sleep and anxiety disorders. Hence, our findings could consist of the healthy spectrum of the association between LC activity and REMS that would lead to insomnia disorder if exacerbated or prolonged over extended periods of time. Future investigations are warranted in a clinical population with, for instance, anxiety and/or insomnia disorder, including tests of memory performance as well as other behavioral measures.

We fortuitously find that a higher LC MRI contrast is associated with better subjective sleep quality. This finding echoes a recent report that lower LC contrast in its middle-caudal portion is linked to a higher number of self-reported nocturnal awakenings in healthy older individuals (Van Egroo et al., 2021a). Although the LC contrast is considered to reflect its structural integrity, its neurobiological bases are still under investigation (Priovoulos et al., 2020). The LC contrast increases over the adult lifespan up to around 55-60y to decrease after (Liu et al., 2019), preventing our understanding on whether a higher LC contrast over time reflects a better or worse situation. In our sample of very healthy individuals, the LC is possibly better preserved (e.g. it may present less tau aggregates (Jacobs et al., 2021)), such that higher contrast is associated with better sleep quality.

According to autopsy data, by the age of 40y, about 100% of the population exhibits some degree of tau protein aggregates in the LC (Heiko Braak and Del Tredici, 2011). The presence of these tau aggregates is likely to affect the LC structure and functioning (van Egroo et al., 2021), and is suspected to contribute to cognitive decline in older individuals (Jacobs et al., 2021). Since we did not assess tau aggregates levels, we cannot address whether tau aggregates contribute to our findings. Similarly, our findings may suggest more prominent associations between the left LC and sleep, however, we do also find associations with the right LC. Since

there is no clear consensus on the potential lateralization of the LC (Poe et al., 2020; Simpson et al., 1997), we have reported here a potential laterality while we do not interpret our findings in terms of lateralization.

Our study has limitations. Most importantly, the timeline of the experiment was different between the younger and older participants. While the baseline night of sleep immediately preceded the fMRI acquisition in the young, a time gap of up to 1 year separates these 2 parts in the older group (see methods). Although sleep undergoes profound changes over the lifetime (Zeitzer, 2013), it is remarkably stable within an individual over a shorter period (e.g. a few months/years) (Tucker et al., 2007). While we acknowledge that the difference in procedure can induce bias, we consider it unlikely to explain the significant association we find in the older, but not in the younger individuals. Our sample also included a larger proportion of woman (73 to 85%) such that differences between sexes, though accounted for in the statistical modeling, could not be properly investigated. Our sample size, while representing a large work effort, remains modest, particularly in the older subsample, and replication is warranted. Future studies may also want to use individually tailored hemodynamic response functions (HRF) to assess LC response. While the canonical HRF we used to model activity over the entire brain seems suitable to model average LC response over a group of participants, individual LC responses can vary substantially across individuals (Prokopiou et al., 2022). Finally, applying an False Discovery Rate (FDR) correction to the p-values of the primary GLMMs, only the association between LC activity and sleep quality remains significant (interaction with the age group) while the association with REMS theta power does not reach the corrected p-value threshold (i.e. $p < 0.016$, see methods), though it may meet the corrected threshold if subject with relatively extreme values are removed. Given that our study is pioneering in seeking relations between sleep features and LC activity during wakefulness *in vivo* in humans, the value of our results remains highly remarkable.

Materials and Methods

Participants

A sample of 52 healthy participants of both sexes, composed of 33 healthy young (age: 22.15 ± 3.27 y, 28 women) and 19 late middle-aged (age: 61.05 ± 5.3 y, 14 women) individuals were recruited from the local community to participate to this study. A summary of the demographic data can be found in Table 1. This study was approved by the faculty-hospital ethics committee of the University of Liège. All

participants provided their written informed consent and received financial compensation.

The exclusion criteria were as follows: history of major neurologic or psychiatric diseases or stroke, a recent history of depression and anxiety, sleep disorders, medication affecting the central nervous system, smoking, excessive alcohol (> 14 units/week) or caffeine (> 5 cups/day) consumption, night shift work in the past 6 months, travel across different time zones during the last 2 months, Body Mass Index (BMI) ≤ 18 and ≥ 29 (for the older participants) and ≥ 25 (for the younger participants), clinical symptoms of cognitive impairment for older subjects (dementia rating scale score < 130; Mini-Mental State Examination score < 27) and MRI contraindications. Due to a miscalculation at screening one older participant had a BMI of 30.9 and one of the younger participants had a BMI of 28.4. Since their data did not deviate substantially from the rest of the sample and BMI was used as a covariate in our statistical models, these participants were included in the analyses.

Protocol

All participants completed an in-lab habituation night under polysomnography to minimize the effect of the novel environment for the subsequent baseline night and to exclude volunteers with sleep disorders (see below). All participants completed a whole-brain structural MRI acquisition and an acquisition centered on the LC using a specific sequence. They further completed the PSQI questionnaire to assess their habitual subjective sleep quality (Buysse et al., 1989). Higher scores are indicative of some sleep difficulties. Participants were requested to sleep regularly for 7 days before the baseline night (±30 min) based on their preferred bed and wake-up times. The compliance was verified by the sleep-wake diary and actigraphy (Actiwatch©, Cambridge Neurotechnology, UK and AX3©, Axivity Ltd, UK). Participants were instructed to abstain from caffeinated beverages and from alcohol as well as to not do excessive physical activity at least three days before the baseline night. The evening before the baseline night participants arrived at the laboratory 4 hours before their habitual bedtime, completed questionnaires including the Beck Anxiety Inventory (BAI) and Beck Depression Inventory (BDI) (A. T. Beck et al., 1988; Beck et al., 1988), and were then kept in dim light (<10 lux) for 3 hours preceding bedtime. Their habitual sleep was then recorded in complete darkness under EEG. Baseline night data was acquired using N7000 amplifiers (EMBLA, Natus Medical Incorporated, Planegg, Germany) and were used for sleep features extraction. All participants completed a fMRI session that included three perceptual tasks,

approximately 3 hours after habitual wake-up time. This paper is centered on the analyses of the oddball auditory task brain responses.

Younger participants completed the fMRI session immediately following the in-lab baseline night. They were maintained in dim light (< 10 lux) between wake-up time and the fMRI session. Older participants were part of a different study (Narbutas et al., 2019; Van Egroo et al., 2019b) and completed the sMRI and fMRI procedures in addition to their initial engagement. Consequently, the baseline nights of sleep and fMRI sessions were completed about 1 year apart (mean ± SD: 15.5 ± 5.3 months). The procedures for the baseline night recordings, including the sleep-wake schedule and light exposures, were identical to those of the young. Prior to the fMRI session participants slept regularly for 1 week (verified with a sleep diary; our experience is that actigraphy reports and sleep diaries do not deviate substantially in older individuals); they were maintained in dim light (< 10 lux) for 45 min before the fMRI scanning.

Sleep EEG metrics

The habituation night included 5 EEG derivations (Fz, Cz, Pz, P3, Oz) while 11 derivations were used for the baseline night (F3, Fz, F4; C3, Cz, C4; P3, Pz, P4; O1, O2), all placed according to the 10–20 system and referenced to the left mastoid (A1) while an electrode was also placed over the right mastoid (A2). Both nights included 2 bipolar electrooculogram (EOG), and 2 bipolar submental electromyogram (EMG) electrodes as well as 2 bipolar electrocardiogram (ECG) derivations. EEG data was digitized at 200 Hz sampling rate. EEG data was then re-referenced off-line to the average of both mastoids using Matlab (Mathworks Inc., Sherbom, MA). Participants with excessive sleep apneas (apnea-hypopnea index ≥ 15) and limb movements (≥ 15/hour) were excluded from the study following the habituation night. No participants suffered from REMS behavioral disorder nor from other parasomnia.

The sleep data were scored in 30-s epochs using a validated automatic sleep scoring algorithm (ASEEGA, PHYSIP, Paris, France) (Berthomier et al., 2007). Arousals and artefacts were detected automatically as previously described (Wallant, 2016) and excluded from the subsequent power spectral density analyses (using Welch's overlapped segment averaging estimator, as implanted in the pwelch Matlab function; 4s epochs without artefact or arousal; 2s overlap). Only frontal electrodes were considered in the analyses because the frontal region is most sensitive to sleep pressure manipulations (Cajochen et al., 1999) and to facilitate interpretation of future large-scale studies using headband EEG, often restricted to frontal electrodes.

Averaged power was computed per 30 min bins, adjusting for the proportion of rejected data (containing artefact/arousal), and subsequently aggregated in a sum separately for REM and NREM sleep as described in (Skorucak et al., 2018). Thus, we computed slow wave energy (SWE) - cumulated power in the delta frequency band during SWS, an accepted measure of sleep need (Plante et al., 2016), and, similarly, we computed the cumulated theta (4-8Hz) power in REM sleep. The cumulated power score would increase with time spent in REMS and SWS, so we included TST as a common covariate in all analyses, as well as REMS duration in secondary analyses.

Auditory oddball task

The task consisted of rare deviant target tones (1000 Hz sinusoidal waves, 100 ms), composing 20% of the tones that were pseudo-randomly interleaved within a stream of standard stimuli (500 Hz sinusoidal waves, 100 ms). The task included 270 auditory stimuli in total (54 target tones). Auditory stimuli were delivered with MRI-compatible headphones (Sensimetrics, Malden, MA). The inter-stimulus interval was set to 2000 ms. Participants were instructed to press with the right index on an MRI-compatible keyboard (Current Designs, Philadelphia, PA) as quickly as possible at the appearance of target sounds. The experimental paradigm was designed using OpenSesame 3.3.8 (Mathôt et al., 2012). The MRI session started with a short session to set the volume of the audio system to ensure an optimal perception of the stimuli.

MRI data acquisitions

MRI data were acquired using a MAGNETOM Terra 7T MRI system (Siemens Healthineers, Erlangen, Germany), with a single-channel transmit coil and a 32-receiving channel head coil (1TX/32RX Head Coil, Nova Medical, Wilmington MA). To reduce dielectric artifacts and homogenize the magnetic field of Radio Frequency (RF) pulses, dielectric pads (Multiwave Imaging, Marseille, France) were placed between the head of the participants and the coil.

BOLD fMRI data were acquired during the task, using a multi-band Gradient-Recalled Echo - Echo-Planar Imaging (GRE-EPI) sequence: TR = 2340 ms, TE = 24 ms, flip angle = 90°, matrix size = 160 × 160, 86 axial 1.4mm-thick slices, MB acceleration factor = 2, GRAPPA acceleration factor = 3, voxel size = (1.4x1.4x1.4) mm^3. The cardiac pulse and the respiratory movements were recorded concomitantly using, respectively, a pulse oximeter and a breathing belt (Siemens Healthineers, Erlangen,

Germany). The fMRI acquisition was followed by a 2D GRE field mapping sequence to assess B0 magnetic field inhomogeneities with the following parameters: TR = 5.2 ms, TEs = 2.26 ms and 3.28 ms, FA = 15°, bandwidth = 737 Hz/pixel, matrix size = 96 × 128, 96 axial slices, voxel size = (2x2x2) mm ^{3}, acquisition time = 1:38 min.

A Magnetization-Prepared with 2 RApid Gradient Echoes (MP2RAGE) sequence was used to acquire T1 anatomical images: TR = 4300 ms, TE = 1.98 ms, FA = 5°/6°, TI = 940 ms/2830 ms, bandwidth = 240 Hz/pixel, matrix size = 256 × 256, 224 axial 0.75mm-thick slices, GRAPPA acceleration factor = 3, voxel size = (.75x.75x.75) mm^3, acquisition time = 9:03 min (Marques & Gruetter, 2013). The LC specific sequence consisted of a 3D high-resolution Magnetization Transfer-weighted Turbo-FLash (MT-TFL) sequence with the following parameters: TR = 400 ms, TE = 2.55 ms, FA = 8°, bandwidth = 300 Hz/pixel, matrix size = 480 × 480, number of averages = 2, turbo factor = 54, MTC pulses = 20, MTC FA = 260°, MTC RF duration = 10000 µs, MTC Inter RF delay = 4000 µs, MTC offset = 2000 Hz, voxel size = (.4x.4x.5)mm^3, acquisition time = 8:13 min. Sixty axial slices were acquired and centered for the acquisitions perpendicularly to the rhomboid fossa (i.e., the floor of the fourth ventricle located on the dorsal surface of the pons).

MRI data pre-processing

EPI images underwent motion correction, distortion correction using Statistical Parametric Mapping (SPM12, https://www.fil.ion.ucl.ac.uk/spm/) and brain extraction using “BET” from the FMRIB Software Library suite (FSL, https://fsl.fmrib.ox.ac.uk) and the final images were spatially smoothed with a Gaussian kernel characterized by a full-width at half maximum of 3 mm.

The background noise in MP2RAGE images was removed using an extension of SPM12 (extension: https://github.com/benoitberanger/mp2rage) (O’Brien et al., 2014). The denoised images were then automatically reoriented using the ‘spm_auto_reorient’ function (extension available from https://github.com/CyclotronResearchCentre/spm_auto_reorient) and corrected for intensity non-uniformity using the bias correction method implemented in the SPM “unified segmentation” tool (Ashburner and Friston, 2005). Brain extraction was then conducted on the denoised-reoriented-biased-corrected image using both the Advanced Normalization Tools (ANTs, http://stnava.github.io/ANTs/) with the ‘antsBrainExtraction’ function and the RObust Brain EXtraction tool (ROBEX, https://www.nitrc.org/projects/robex) (Iglesias et al., 2011). The method yielding to the best extraction for each individual, as assessed by visual inspection, was used for

subsequent steps. A whole-brain T1 group template was created using ANTs, based on preprocessed MP2RAGE images of all subjects except for one, the MP2RAGE image of whom was not suitable due to a bad positioning of the field of view during the acquisition. Finally, the preprocessed MP2RAGE image of each subject was normalized to the Montreal Neurological Institute (MNI) space (with a 1x1x1 mm^3 image resolution). The purpose of using a template that is specific to our dataset was to improve the registration into the MNI space using a study specific intermediate space. The transformation parameters obtained from normalization were later used for registering first-level statistical maps into the MNI space to conduct group-level fMRI analyses.

To extract the LC contrast, T1 structural images in subject space (after removing the background noise) were up-sampled by a factor 2 [(0.375x0.375x0.375) mm^3] to avoid losing in-plane resolution when registering the LC slab to the T1 image. The up-sampling was done using the 'nii_scale_dims' function from an extension of SPM12 (extension: https://github.com/rordenlab/spmScripts). The complete LC contrast extraction was done in the original subject space. The MT-TFL image of each subject was registered with the whole brain up-sampled T1 image by means of a two-step process: (i) an approximate manual registration to extract the parameters for an initial transformation using ITK-SNAP (Yushkevich et al., 2016), and (ii) an automatic affine registration based on the initial transformation parameters, using ANTs. MT-TFL data of one young subject was not usable, due to the excessive motion of the participant, leading to a registration failure.

The LC appearing hyperintense on registered MT-TFL images was manually delineated by two expert raters and the intersection of the LC masks of the two raters was computed as the final LC mask for each individual. The LC mask was skeletonized by only keeping the voxel with the highest intensity in each axial slice. Based on the skeletonized LC mask, the LC contrast was computed after normalization of each LC slice intensity to a slice-corresponding 2D reference region (a 15 x 15 voxels region, corresponding to a (5.5 x 5.5)mm^2 square region) situated anteriorly (and centrally) in the pons, in the pontine tegmentum. For example, the left LC contrast was defined as:

$$Contrast\ LC_{left} = \ mean\left(\frac{LC_{left_i} - mean(2D\ pons_i)}{mean\ (2D\ pons_i)}\right)$$

Where:

- i is the slice index along the (left) LC,

- $LC_{Left,}$ is the intensity of the voxel with the highest intensity in the axial slice with index i,

- mean (2D $pons_i$) represents the mean intensity in the 2D reference region corresponding to the axial slice with index i.

The LC contrast was computed as the mean LC contrast between the left and right LC. The distribution of the LC contrasts across individuals was investigated by computing the Probability Density Function (PDF), using a kernel density nonparametric method (ks density MATLAB R2021a built-in function). Individual skeletonized LC masks were used for extracting the LC activity during the oddball task in the subject space. To investigate the activation of the LC at the group level, an LC group-wise template was created. The LC mask of each volunteer was normalized to the structural group template, and then to the MNI (MNI152 - 1x1x1mm^3). This was done using the 'antsApplyTransforms' ANTs command, with the transformation parameters estimated (i) when registering the subject-specific MP2RAGE image to the structural template, and (ii) the transformation parameters estimated when registering the structural template to the MNI. The final LC group-wise template was created as the sum of all masks.

Statistical Analyses

Statistical analyses were conducted using SPM12. A high-pass filter with a 128 s cutoff was applied to remove slow signal drifts. The timing vector with the appearance of the target tones was convolved with the canonical HRF to model the event-related response and was used as the main condition in a General Linear Model (GLM). The PhysIO Toolbox (https://www.tnu.ethz.ch/en/software/tapas/documentations/physio-toolbox) was used to compute physiology-related voxel-wise signal fluctuations based on respiratory and cardiac pulsation data (Kasper et al., 2017), that was available in 48 volunteers (physiological data was not available for 4 volunteers). The Fourier expansion of cardiac and respiratory phase, 14 parameters computed with the toolbox, as well as the 6 realignment parameters were used as multiple regressors of no interest in the GLM. The first-level statistical analysis was conducted in the subject space.

The mean functional image was registered to the MP2RAGE image to extract the corresponding transformation matrix used to register the first-level statistical map of each subject to the structural image. Therefore, for all subjects, statistical maps

corresponding to the appearance of target sounds were registered to the native space, normalized to the group template space and then to the MNI space. A second-level analysis was then conducted in the MNI space, where age, sex and BMI were used as covariates. The group-wise mask of the LC was used to assess specific activation of the LC. Due to the small size of the nucleus, LC activation was not expected to survive stringent whole-brain FWE correction. Therefore, a small volume correction using the LC template was conducted using SPM12 to detect voxel-level $p<.05$ FWE- corrected results within the LC mask.

REX Toolbox (https://web.mit.edu/swg/software.htm) was then used to extract the activity estimates (betas) associated with the appearance of the target sounds in the LC mask of each subject, within the subject space (Duff et al., 2007). This procedure ensured that any potential displacement and bias introduced by the normalization step into the common MNI space did not affect individual activity estimates. Statistical analyses using these activity estimates were performed in SAS 9.4 (SAS Institute, NC, USA). Analyses consisted of Generalized Linear Mixed Model (GLMM) with sleep features of interest as the dependent variable, the LC activity as an independent variable and age group (younger, older), sex, BMI included as covariates, and subject as a random factor. GLMM were computed according to the distribution of the dependent variable. In the primary analyses, we tested 6 independent GLMMs, and to account for multiple comparisons, we applied the Benjamini–Hochberg procedure for false discovery rate correction of the p-values using and online tool https://tools.carbocation.com/FDR. The tool yielded a corrected p-value of 0.016.

The mediation analysis was computed using CAUSALMED procedure in SAS including bootstrap confidence interval computation. Subjective sleep quality was the dependent variable with a direct pathway to LC activity and an indirect pathway mediated by theta power in REMS, which was squared root to satisfy the parametric assumption of the procedure. An interaction effect between theta power in REMS and LC activity was included, while age, sex and BMI were used as covariates. The percentage of controlled direct and pure indirect effects are reported together with their associated p-values.

Optimal sensitivity and power analyses in GLMMs remain under investigation (e.g. (Kain et al., 2015)). We nevertheless computed a prior sensitivity analysis to get an indication of the minimum detectable effect size in our main analyses given our sample size. According to G*Power 3 (version 3.1.9.4) (Erdfelder et al., 2009), taking

into account a power of 0.8, an error rate α of 0.05, and a sample size of 52 (33 + 19), we could detect medium effect sizes r > 0.33 (one-sided; absolute values; CI: 0.06–0.55; R^2>0.11, R^2 CI: 0.003–0.3) within a linear multiple regression framework including one tested predictor (LC activity) and three/four covariates (group, sex, BMI, and TST where relevant).

General discussion

Preamble

Mechanisms underlying sleep deterioration in healthy and pathological aging remain enigmatic. Human research has demonstrated that aging interferes with the ability to produce deep restorative sleep, and this may causatively contribute to the development of the age-related increase in ID prevalence as well as to neurodegenerative disorders, including AD.

It is known that AD pathological processes, such as the gradual build-up of Aβ plaques and tau neurofibrillary tangles, begin many years before any observable clinical signs of cognitive impairment (Jack & Holtzman, 2013). For AD diagnosis, these biomarkers are considered jointly together with the degree of neurodegeneration and regionally specific reduction of cerebral glucose metabolism, synaptic dysfunction, and mitochondrial dysfunction (Kumar, Singh, & Ekavali, 2015), which alone may also be regarded as part of normal aging (Bishop, Lu, & Yankner, 2010). The medications slow the disease's course and alleviate symptoms, but they do not completely cure the condition, probably because, although the neuropathological characteristics of AD are well known, the intricate details of the mechanism are still unclear. Furthermore, poor sleep and insomnia in older age is a recognised risk factor for AD (Sadeghmousavi, Eskian, Rahmani, & Rezaei, 2020). It has been demonstrated that ID patients have increased Aβ42 levels and that both Aβ40 and Aβ42 levels are correlated with subjective sleep quality, measured by the PSQI (Chen, Wang, Zhang, Wang, & Gao, 2018).

ID pathophysiology is however even more elusive than that of AD, and there is still no widely acknowledged model for its aetiology, despite recent advancements in this area. This might be related to the heterogeneity of insomnia (Benjamins et al., 2017), its highly comorbid nature (Wittchen et al., 2011), or different levels of study, from phenomenology to physiology. Recent research indicates considerable involvement of heritability in the pathophysiological processes of ID; genes associated with sleep-wake processes, brain functioning and arousal regulation have been linked to ID. The complicated interplay of these genes may account, at least in part, for the variation in ID symptoms (Levenson, Kay, & Buysse, 2015).

The mystery of neurobiological basis for ID may get closer to be solved when considering factors contributing to vulnerability to develop ID. These factors include genetic variants, early life stress, major life events, and brain structure and function. The genetic architecture of the ID, being an important contributor to the disease etiology, can be investigated in a number of protocols including twin and family

studies, candidate gene studies, and GWAS (Lind & Gehrman, 2016). In our work we took advantage of the results of a GWAS study to assess the genetic risk for ID by using PRS.

The high heritability of both AD and ID allowed us to experimentally investigate early factors that can affect sleep and contribute to disease vulnerability. We addressed the question whether the genetic predisposition for AD and ID (the factor that starts its action from conception) is already associated with sleep characteristics in young people without any sleep complaints or signs of cognitive decline. The approach employed in the two studies combined genetic risk assessment with electrophysiological and self-reported sleep phenotypes in healthy young individuals. Interestingly, according to previous research, the AD-related sleep alterations derived from EEG, including reductions in total sleep time, sleep efficiency, percentage of SWS and REM sleep, increases in sleep latency, wake time after sleep onset, number of awakenings and REM latency, as well as EEG frequency components (Ye Zhang et al., 2022), closely match those of ID (Baglioni, Regen, et al., 2014)), which provides indirect evidence for partly common pathways underlying AD and ID. It was fascinating to discover that even at younger age, normally sleeping adults *do* differ with regard to their liability for ID / AD, and that the sleep characteristics involved in the associations with ID / AD PRS do not directly coincide.

PRS for ID and AD associations with sleep

Heterogeneity of PRS for ID and AD in association with slow wave energy

Our results in chapters 3-4 may seem contrasting with each other at a first glance, if we posit that ID and AD have common mechanisms: the higher cumulated SWA during NREM sleep was associated with higher PRS for AD but with lower PRS for ID. Interestingly, the link is not only limited to delta waves during NREM sleep: PRS for ID was associated with the slow rhythm that included activity in delta and theta bands in both NREM and REM sleep, and PRS for AD was associated with cumulated total power between 0.5 and 25 Hz during both NREM and REM sleep; this frequency range non-specificity may originate from overall individual variability in EEG amplitude, and the larger amplitude might be indicative of a more synchronized brain activity. In addition, the higher daytime sleepiness was associated with higher PRS for AD while higher probability of falling asleep during the day was associated with lower PRS for ID. However, considering the complex nature of the AD/ID

pathogenesis, these results provide food for thought and invite constructive speculation. Hereafter we provide arguments *for* the 'contradictory' nature of the aforementioned results as well as arguments *against* it.

Firstly, it is widely accepted that AD shares a strong genetic basis with ID (Monereo-Sánchez et al., 2021). At the same time, while ID has common genetic aetiology with depression (O'Connell et al., 2021; Purves et al., 2020; Stein et al., 2018), a recent research on AD and depression yielded a substantial genetic overlap, with *mixed direction of effects*, between AD and depression (Monereo-Sánchez et al., 2021). Altogether, this may provide indirect evidence that a substantial number of genetic common variants associated with AD and ID have opposite effects and corresponding contribution to PRS for AD and ID. Following this reasoning, we looked if the PRS for AD and ID were negatively correlated in our cohort, but we did not find associations between the PRS ($p = 0.7$: we used those that showed the strongest associations with sleep phenotype, i.e. including all SNPs for PRS for AD and SNPs thresholded at $p=1$ for PRS for ID). Neither we found any associations of AD/ID PRS with depression / anxiety indexes, so we concede that the genetic architecture of AD and ID and its relation with sleep phenotypes is complex and cannot be viewed as just having positive or negative impacts.

Secondly, APOE-ε4, constituting the highest genetic risk for sporadic AD, has been linked to insomnia symptoms: the risk of sleep disturbances, more specifically WASO, but not the number of awakenings, was increased, while the subjective sleep quality (assessed with PSQI) did not differ in the APOE-ε4 carriers compared to non-carriers (Drogos et al., 2016). A recent study however indicates that APOE-ε4 affects sleep by mechanisms *independent* of AD pathological change: APOE-ε4 homozygosity was associated with a composite score of sleep disturbance which included increased latency, increased wake time after sleep onset, wandering, early morning awakening and excessive daytime sleep; a subsample analysis showed that APOE-ε4 heterozygosity was similarly associated in individuals without dementia (Blackman, Love, Sinclair, Cain, & Coulthard, 2022).

Furthermore, SWS and SWA reduction is indeed a strong marker of age-related neurodegenerative diseases, being a key component of the association between sleep–wake regulation and Aβ aggregation (Branger et al., 2016; Ju et al., 2017; Mander et al., 2015; Varga et al., 2016); also tau pathology has been recently linked to SWA (Lucey et al., 2019). SWA disruptions are present in healthy aging, but more pronounced in older adults suffering from cognitive decline. The two potential

mechanisms that link SWA reduction and AD pathology are structural degradation and impaired metabolic clearance (Wunderlin, Züst, Fehér, Klöppel, & Nissen, 2020). Considering this, one could expect lower cumulated SWA (namely SWE in our paper) in people at higher genetic risk for AD. However, various AD phenotypes can show different sleep architecture (Wunderlin et al., 2020), and at the group level these differences may be blunted. What's more, the genetic component assessed with PRS based on DNA remains stable over the lifespan while physiological and behavioral characteristics are highly plastic and one aspect of this plasticity is changes over age. The participants composing the cohort for our analysis are very young, and their sleep patterns may change dramatically when they will reach the age of true susceptibility to AD.

Finally, insomnia is also characterised by reduced SWA and SWS (Pigeon & Perlis, 2006), however the question if this reduction shares the origin with that of AD, remains open. A recent work investigated PSG in people with AD and ID vs those matched by age with ID only. Individuals with AD & ID had less SWS and lower EEG power, particularly in the lower-frequency bands (SWA) than those with ID, which means that the AD-related SWA and SWS changes are more profound in AD patients than in controls even controlling for the presence of ID (Wunderlin et al., 2020), demonstrating ID-independent SWA disruption in AD.

Limitations

A number of limitations of these two works have already been reported in the discussions of the corresponding chapters, and here we will provide some additional general considerations.

Firstly, our cohorts consisted of quite homogeneous community-based sample which (probably) allowed us to find the small-sized effects. This might nevertheless affect the generalisability of our findings, so a replication study in another cohort, including for example more women or another age range for example, could only be beneficial. Noteworthy, the portability of sleep-related PRS, including PRS for ID, across cohorts was illustrated on UK Biobank data and Finnish cohort (Perkiö et al., 2023).

Secondly, while we potentially have isolated the core characteristics of sleep important for AD and ID which are not yet affected by age, comorbidities and medications, we should keep in mind that this in turn again could have limited the potential of generalisability. Heritability of some behavioural measures, e.g. general

cognitive ability, linearly increases with age (Haworth et al., 2010) indicating a complex gene-environment interplay: as we get older, we increasingly select, modify and create our own experiences in part on the basis of our genetic propensities (Plomin, 1994). Whilst the heritability of AD and ID would not really change with age, given that AD (and ID to some extent) is a binary measure in its relation to an individual (we either have it or not, contrary to cognitive ability which can be estimated gradually), however there can be both intrinsic and extrinsic environmental factors that would come into play and alter profoundly variability in sleep characteristics in a *slightly older* cohort.

In addition, and following the previous point about gene-environment interaction, we of course cannot predict if the individuals from our cohort would develop ID or AD, but this question is out of the scope of our study, and could be investigated only longitudinally – and over very long periods of time, particularly for AD.

Potential directions

Our methods for PRS calculation were quite common and robust at the time when we completed the analyses, however new approaches that use model linkage disequilibrium supersede those using simple p-value thresholding (e.g. LDpred (Vilhjalmsson et al., 2015)), and if a replication is considered, the up-to-date methods should be used. It has been recently shown that a small number of SNPs better predict AD (Q. Zhang et al., 2020), while our most significant result was obtained by taking the greatest number of SNPs, which again speak in favour of using more modern approach for PRS calculation.

Besides, the risk for ID/AD and other sleep traits which are highly heritable and related to diseases, may be assessed and linked to cognitive function. For example, recently higher PRS for insomnia was linked to lower global cognitive function and higher risk of mild cognitive impairment (MCI) in a large cohort (N>16 000 participants aged 18–74); higher PRS for daytime sleepiness was likewise linked to increased MCI risk (Yuan Zhang et al., 2023). As to our analysis, we also tested links between ID / AD PRS and cognitive measures, but we did not find any correlations likely due to the young and narrow age range of our group, as well as to our samples size. Furthermore, sleep duration and sleep quality are both largely genetically determined (Kocevska, Barclay, Bramer, Gehrman, & Van Someren, 2021), and interestingly, sleep duration PRS was heterogeneously associated with MCI risk

among short, normal and long sleepers, suggesting that genetic predisposition to long sleep might reflect pathophysiological processes related to sleep-wake control and general health (Yuan Zhang et al., 2023). Besides, it was reported that higher sleep duration PRS is associated with more rapid decline of visuo-spatial abilities over time (Tsapanou et al., 2021). However, in an earlier study, higher sleep duration PRS was linked to better cognitive performance (Tsapanou et al., 2020), and the authors explain this contraversion by the fact that this association was driven by younger group while the above-mentioned results are from older adults.

Additionally, big cohort open access data (e.g. UK Biobank (Katori, Shi, Ode, Tomita, & Ueda, 2022)) may be used to test associations between sleep and genetic liability for diseases. Although sleep phenotyping in a big cohort is typically quite limited and is based either on the actimetry data or self-report, certain sleep phenotypes may benefit from long-term assessment, for which simple yet valid sleep measurement is required. For example, by using a sleep/wake classification algorithm, Katori et al. revealed 16 clusters, including seven different insomnia-like phenotypes (Katori et al., 2022). Lane et al. used UK Biobank data to confirm the effects of 57 loci for self-reported insomnia symptoms on actimetry-derived measures of sleep efficiency and sleep duration (Lane et al., 2019). In our analyses, we tested the associations between ID PRS and actimetry-derived sleep-wake fragmentation index but we did not see a link, potentially because of the moderate sample size. Quantitative characterization of sleep phenotypes and their relation to PRS of sleep disturbances / traits might increase our understanding of the biological mechanisms including genetic influences underlying human sleep variation.

The LC and sleep

Pitfalls of sleep research over the lifespan

The difficulty of studying mechanisms of both AD and ID in their relation to normal sleep is mainly due to two reasons. First, both AD and ID may be considered as age-related conditions (as age is the main risk factor for AD, and ID prevalence is substantially higher in older people) and aging is a complex process, and quite commonly it is difficult to disentangle its healthy form from the diseases which accompany it, as well as from effects of medications. In addition, as mentioned before, the pathological basis for ID is not well defined.

Second, while cellular and molecular mechanisms are generally investigated in animals, the results of animal studies on aging can be only limitedly translated to human research: direct comparisons between species are not always simple, particularly when it comes to traits as complicated as sleep. More specifically, despite of the wide use of animal models in sleep and aging studies, evidence from rodents lacks consistency and often contrasts with that from humans. For example, recent laboratory rodent data shows that, in contrast to human beings, sleep duration as well as EEG SWA during NREM sleep increases with aging (Panagiotou, Vyazovskiy, Meijer, & Deboer, 2017), which may be related to an increased homeostatic sleep need in older animals or impaired ability to dissipate the increased sleep pressure (Panagiotou et al., 2017). The differences in sleep changes may be due to the shorter lifespan of rodents compared to humans (Panagiotou et al., 2017), but a more sophisticated explanation may consist of interaction of their specific evolutionary history and ecological and physiological demands: the dynamics of sleep across the lifespan might reflect both the capacity for environmental adaptation and age-dependent changes in the neurobiological bases of sleep (McKillop & Vyazovskiy, 2020). Besides, studying human AD in rodents models may be complicated by the fact that rodents lack a human-like cortex while the structures that are vulnerable to the AD-related process are mostly the cerebral cortex itself or subcortical nuclei that diffusely project to and enhance 'higher' CNS functions (Braak & Del Tredici, 2015; Hitziger, 2015). In addition, most wild type laboratory rodent model lack tau NFT and Ab plaque which are only found in genetically engineered animals.

Furthermore, human insomnia is a multi-factorial condition and can be only partially reproduced in animal models. Rodent sleep nonetheless shares several essential characteristics with human sleep, including homeostatic regulation, circadian distribution, sensitivity to endogenous and external variables, and reactivity to medicines that promote wakefulness or sleep. These characteristics serve both for reproduction of insomnia in animals and theoretical models in humans (Revel, Gottowik, Gatti, Wettstein, & Moreau, 2009; Richardson, 2007). Interestingly, one group of experimental situations holding the potential for use as models of insomnia consist of models derived from diseases and pathophysiological conditions, including AD. Sleep disturbances similar to those in AD patients, such as reduction of NREM and REM sleep, increased fragmentation of sleep, arise in several mouse models of AD. However, it is only when mechanisms causing insomnia will be resolved in

humans that a fully accurate reproduction of primary insomnia in animals may become feasible.

Genetics, sleep and LC

In the meanwhile our study and findings suggest an intriguing perspective of looking at ID and AD: we used genetics as a proxy measure to assess the disease vulnerability potentially reflected in the sleep variability. We further hypothesized that the disease vulnerability may as well originate from the LC, which is a key player in sleep-wake regulation. The LC is also one of the first sites of tau neuropathology and a theoretical trigger of arousal accumulation that through a chain of neuronal events leads to ID. In other words, we sought to verify that the factors intrinsically related to the disease, in this case genetic liability and the LC functionality, might be reflected in broadly speaking sleep quality many years before any detectable neuropathology or easily observable sleep disturbance. Based on our assumption that inherent LC deterioration / malfunction captured by the MRI scanner would equivalently affect wakefulness and sleep, we found that the LC functioning is indeed related to subjectively evaluated quality of sleep in older people; moreover, we showed that higher LC activity is related to lower theta power generated during REM sleep in the same sample of older individuals. With regards to PRS studies, among other things, we showed that at a young age, lower theta power during REM sleep is correlated with higher PRS for ID, but (potentially) with lower PRS for AD. Further speculation can therefore be added to the framework of the LC constituting a bridge between sleep-wake dysregulation and AD (Van Egroo, Koshmanova, Vandewalle, & Jacobs, 2022), as well as in the framework of ID model suggested by Eus Van Someren (Van Someren, 2021). The outcomes of the three studies described in this thesis taken all together suggest that the genetic predisposition for ID and AD could contribute to the LC structural / functional properties (as well as to other structures involved in sleep generation and modulation of course). The result of this contribution would be detectable at the age when sleep variability becomes prominently important for the general health status (Briones et al., 1996; Dalmases et al., 2019; Sahraian, Javadpour, & Mani, 2011), and when sleep alterations have higher chances to develop into a risk factor for sleep disorders and neurodegeneration. An ideal study to test this would include longitudinal assessments of sleep parameters and LC functioning over the entire adulthood lifespan or across each decade to see how the associations between sleep, LC and predisposition for AD/ID evolve. However, subtle

signal from common genetic variants even if collected into more powerful PRS, requires large sample size to detect small effects typical of genetic studies. A cohort of at least several hundreds of individuals would therefore be mandatory. It looks hardly feasible for a single laboratory, taking into account the costs of MRI scanning and man power required. Multi-centric studies will be needed and may prefer ambulatory in-home sleep recordings device to strict laboratory protocols, even if the collected data may not be as rich. Below we describe some more plausible research scenarios.

Future prospectives

It has been proposed that the vulnerability to develop ID employs rather the brain circuits involved in arousal and emotion regulation than those responsible for circadian or homeostatic sleep regulation. An increasing amount of reports convey to the fact that both subjectively and objectively reported deviations in sleep are underlined by hyperarousal in ID (Feige et al., 2008). The insufficient silencing of the LC during REMS would work in concert with the above-mentioned factors and lead to insufficient overnight adaptation to emotional distress, the core mechanism of ID (Van Someren, 2021). The direct way to test this model would be to image the LC activity in humans during the night in insomnia patients and compare it to controls and then look if the LC silencing is more profound in the control group. However, this is a challenging objective: sleeping an entire night in the scanner (and with EEG) would be close to impossible: existing EEG/MRI studies usually collect only incomplete sleep cycles during which naturally occurring REM sleep is not reached while inside the scanner. A more feasible approach that was used in our fMRI study is to assess the LC properties during wakefulness and see if they are associated with sleep. We investigated this in healthy individuals without sleep complaints which therefore could not be classified as insomnia complaints. This could appear as a limitation of the study, but on the other hand, the general goals of the project included unraveling sleep features associated with the LC functioning before any potential ID / AD onset, in an attempt to disentangle ID / AD vulnerability from aging and comorbidities, such as anxiety, major depressive and posttraumatic stress disorders which may have overlapping underlying mechanisms. Our results yielded a link between sleep quality, REM sleep essential oscillation power and LC activity. We did not find a link between LC activity and restless REM sleep characteristics, for example arousals in REM sleep, which are prominent in ID patients. It would be

interesting to do a similar protocol on insomnia patients in which one could find more links between REM sleep and LC. This would allow seeking if the LC functioning is associated with the phasic events defining restless REM sleep: more arousals / awakenings / eye movements during REM sleep and with other electrophysiological signatures of hyperarousal which can be observed during sleep (e.g. more fast frequencies in the EEG signal (Feige et al., 2013)) and wakefulness (e.g. less alpha and more beta activity (Colombo et al., 2016; Freedman, 1986)). Furthermore, the LC activity may be indirectly assessed by probing overnight changes in functional connectivity of fMRI brain responses to emotional stimuli in insomnia patients and controls. The rationale for this originates from the combining results of previous studies and the above mentioned insomnia model. FMRI studies assessing BOLD responses underlying task performance remain scarce and rather inconsistent in ID research (Tahmasian et al., 2018), however stronger instant activation in the amygdala (a major part of the emotional brain network), as well as in regions of the salience network, including limbic regions and the anterior cingulate cortex, was revealed in response to emotional stimuli in insomnia patients compared to controls (Baglioni et al., 2010; Baglioni, Spiegelhalder, et al., 2014; Wassing, Lakbila-Kamal, et al., 2019; Wassing, Schalkwijk, et al., 2019). The LC connectivity with these regions may be indicative of overnight adaptation to emotional distress, linked to the restless REM sleep and provide new insights on the neuronal basis for ID. Particular attention should be allocated to the influence of confounding factors such as depression or anxiety levels / comorbidities as they may have similar mechanisms (Van Someren, 2021) and at least partly originate from hyper-responsivity of the LC-NE system in sustained stress responses (Grueschow et al., 2021).

Future studies may seek to characterize the relationships between the LC structural/functional properties and sleep-wake regulation across the human lifespan. It would be interesting to look at the links between LC-related MRI metrics and sleep-wake dimensions in different age groups. Various tasks may be used for assessment of LC functioning. Interestingly, in our lab, a perceptual rivalry task yielded results contrasting to the ones of Chapter 6: higher LC activity during this visual task is associated with higher theta power in REM sleep, which provides complimentary but complicated picture and needs further investigation. Besides, coupling of sleep spindles to slow waves have been linked to AD-related processes including Aβ burden and longitudinal memory decline in late middle aged individuals (Chylinski et al., 2022), and similar methodology exploring sleep from microstructural point of view may be employed to look into LC contribution to sleep.

Another line of research could directly investigate the LC functional/structural properties in the context of AD and ID. A recent study by Elman et al. showed that reduced MRI-assessed LC-NE contrast is associated with daytime sleep-related dysfunction, poorer cognitive function which in turn both increased the risk for mild cognitive impairment (Elman et al., 2021). Besides, it was demonstrated that lower integrity of the middle-to-caudal LC, is associated with more frequent self-reported nocturnal awakenings in cognitively unimpaired older adults, particularly in individuals with higher plasma levels of total tau protein burden (Van Egroo, van Hooren, & Jacobs, 2021). Furthermore, in the context of AD, additional animal work relating glymphatic clearance of toxic proteins to fluctuations of LC-NE activity across the sleep-wake cycle would provide valuable insights into the potential pathways linking the LC-NE system to AD pathogenesis through sleep-wake dysregulation. Indeed, in their seminal work on the role of the sleep-wake cycle for metabolite clearance in mice, Xie et al. demonstrated that elevated NE levels impaired glymphatic function by restricting the interstitial space volume (Xie et al., 2013), but no direct assessment of LC-NE activity was available in that study. In turn, existing optogenetic studies modulating LC-NE activity across the sleep-wake cycle have not evaluated its impact on glymphatic clearance, nor did they investigate the long-term effect of chronic LC-NE photoactivation/photoinhibition on Aβ or tau protein burden in AD mouse models. In humans, clear-cut evidence and methods to measure such a sleep-dependent glymphatic clearance system are still lacking (Mestre, Mori, & Nedergaard, 2020), although one recent study by Fultz et al. provides support to the existence of a temporally ordered sequence of events during sleep in the human brain, linking the occurrence of slow waves to CSF flow through hemodynamic oscillations (Fultz et al., 2019).

Additional longitudinal designs with repeated cognitive, sleep-wake, and LC MRI assessments in cognitively impaired and unimpaired older individuals will be crucial to shed light on the temporal ordering of events, and will contribute to understanding the isolated protective effects of preserved sleep-wake regulation (Shi et al., 2018) and LC-NE integrity (Mather & Harley, 2016) for cognitive performance in aging and AD.

In addition, interventional studies interfering with sleep-wake regulation and/or modulation of LC-NE activity through pharmacological means or non-invasive stimulation techniques will be critical to address causality in the aforementioned relationships. Indirectly bolstering LC-NE function with transcutaneous vagus nerve

stimulation has been proposed to harbor some positive effects on subjective sleep quality in one study of older individuals (Bretherton et al., 2019), but objective evaluation of its quantitative impact and its long-term effects on the sleep-wake cycle, potentially in combination with other sleep-wake interventions such as sleep hygiene education or cognitive behavioral therapy for insomnia, remains to be performed.

In turn, the deleterious effect of fragmented sleep-wake periods on the structural integrity of the LC-NE system proposed in animal studies (Owen et al., 2021; Zhu et al., 2018) remain to be established in humans. Studies including clinical sleep interventions in populations with different degrees of sleep-wake fragmentation, such as elderly individuals, frequent nappers, or sleep apnea patients, would thus provide valuable knowledge for further development and evaluation of preventive interventions in populations at higher risk of cognitive decline and AD trajectories.

Finally, because ID is characterized by the restless REM sleep provoked by insufficient silencing of the LC, the most direct approach to pharmacologically treat ID by reducing these adverse consequences of LC over-function would be the use of medication that either blocks excitatory NE receptors (e.g., β-blockers and α1-antagonists) or decrease the release of NE (α2-receptor agonists). There are reports indicating the enhancing of sleep quality while using a particular β-blocker, Nebivolol (Yilmaz et al., 2008), but other data is contradictory and shows that β-blockers side effects may have adverse and include suppression of REM sleep rather than enhancing its consolidation; clinical trials testing the effects of α1-antagonists and α2-receptor agonists also yielded conflicting evidence on sleep quality enhancing and REM sleep amelioration, however there are no studies reported on ID yet (Van Someren, 2021).

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Appendices

Appendix 1

The full text of the review published in the journal Sleep Medicine Reviews in 2022.

Importance of the locus coeruleus-norepinephrine system in sleep-wake regulation: implications for aging and Alzheimer's disease

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Running head: Locus coeruleus, sleep, and Alzheimer's disease

Summary

Five decades ago, seminal studies positioned the brainstem locus coeruleus (LC) norepinephrine (NE) system as a key substrate for the regulation of wakefulness and sleep, and this picture has recently been elaborated thanks to methodological advances in the precise investigation and experimental modulation of LC structure and functions. This review presents and discusses findings that support the major role of the LC-NE system at different levels of sleep-wake organization, ranging from its involvement in the overall architecture of the sleep-wake cycle to its associations with sleep microstructure, while accounting for the intricate neuroanatomy surrounding the LC. Given the particular position held by the LC-NE system by being at the intersection of sleep-wake dysregulation and initial pathophysiological processes of Alzheimer's disease (AD), we conclude by examining emerging opportunities to investigate LC-NE mediated relationships between sleep-wake alteration and AD in human aging. We further propose several research perspectives that could support the LC-NE system as a promising target for the identification of at-risk individuals in the preclinical stages of AD, and for the development of novel preventive interventions.

Keywords: Locus coeruleus; Norepinephrine; Sleep-wake regulation; Sleep microstructure; Human aging; Tau pathology; Alzheimer's disease

Abbreviations:

5-HT	serotonin
ACh	acetylcholine
AD	Alzheimer's disease
Aβ	beta-amyloid
BOLD	blood-oxygen level dependent
DMH	dorsomedial hypothalamic nucleus
EEG	electroencephalography
FTG	gigantocellular tegmental field
LC	locus coeruleus
LDT	laterodorsal tegmental nucleus
MCH	melanin-concentrating hormone
NE	norepinephrine
NREM	non-rapid eye movement
PPT	pedunculopontine nucleus
PSP	progressive supranuclear palsy
PTSD	post-traumatic stress disorder
REM	rapid-eye movement
SCN	suprachiasmatic nucleus
TMN	tuberomammillary nucleus
VLPO	ventrolateral preoptic area
VTA	ventral tegmental area

Introduction

From young adulthood to older age, several macro- and microstructural aspects of sleep and wakefulness are altered so much that it may constitute one of the most evident behavioral and physiological correlates of human aging (Mander et al., 2017). With increasing age, sleep becomes shallower and shorter, and the overall sleep-wake cycle appears more fragmented, as evidenced by the frequent intrusion of wakefulness periods during sleep (*i.e.* nocturnal awakenings) in older individuals (Mander et al., 2017). Over the past decade, a growing body of evidence has established that such changes in wakefulness and sleep contribute to the pathophysiological mechanisms of neurodegenerative diseases including Alzheimer's disease (AD), which often involves what could be considered as an exacerbated form of age-related sleep-wake dysregulation (Van Egroo et al., 2019c). Interestingly, the majority of the nuclei controlling sleep and wakefulness are affected by neuropathological processes early in the course of AD, before the onset of cognitive symptoms (Lew et al., 2021). Among them, the brainstem locus coeruleus (LC)-norepinephrine (NE) system is receiving increasing attention, owing to a series of recent advances in the precise investigation of its structure and function (Poe et al., 2020). Crucially, these scientific breakthroughs provide researchers with a new theoretical framework which sets the LC-NE system as a nexus between early sleep-wake disruption in aging and AD pathogenesis.

Many questions remain unanswered, however, with regards to the exact contribution of the LC-NE system to the regulation of sleep and wakefulness and their associated features, especially in humans as illustrated by the scarce number of *in vivo* studies. Here, we will start by introducing the structure and functions of the LC-NE system in the context of the broader sleep-wake circuitry. We will then present evidence supporting the important role of the LC-NE system in the overall organization of the sleep-wake cycle. Next, we will discuss the involvement of the LC-NE system in sleep macrostructure, *i.e.* in non-pathologic transitions across sleep stages from non-rapid eye movement (NREM) to rapid-eye movement (REM) sleep, with an emphasis on the specific relationships between LC-NE and REM sleep. Recent findings of direct associations with microstructural aspects of sleep will also be considered. We will end with emerging questions and perspectives regarding the potential utility of investigating LC-NE mediated sleep-wake alteration in aging and AD.

The locus coeruleus-norepinephrine system: structure, functions, and proxy measures

The LC is a nucleus located bilaterally in the dorsal area of the rostral pons, lateral to the fourth ventricle, extending from the lower level of the inferior colliculus to the motor nucleus of the trigeminal nerve (Sharma et al., 2010). The name *locus coeruleus* (Latin for 'blue spot') originates from the blue color observed during the initial histological investigation of this brain structure, which is due to the presence of neuromelanin granules within LC NE-containing neurons. Despite its small number of neurons and its modest size (~14.5 mm long and ~2.5 mm thick in adult humans) (Beardmore et al., 2021), the LC constitutes the primary source of NE for the central nervous system (Poe et al., 2020). LC neurons possess

immensely ramified axons that allow for extensive projections and release of NE over the whole brain, including the hippocampus, amygdala, thalamus, and neocortex (**Fig. 1**), with the exception of a few dopaminergic basal nuclei (Schwarz et al., 2015). Mixed findings were reported with regards to age-related effects on LC-NE structural alteration in *post-mortem* investigations of non-pathological aging, whereas several *in vivo* studies suggest an inverted U-shape curve between MRI-assessed LC signal and increasing age, with the 5th decade as a tipping point (Beardmore et al., 2021; Liu et al., 2017). By contrast, LC-NE neurodegeneration is clearly evident in neurodegenerative conditions, such as AD, with patients consistently displaying LC neuronal loss compared to controls, as early as in the prodromal stages of the disease (Beardmore et al., 2021).

Three families of receptors (α_1-, α_2-, and β-adrenergic receptors), with either excitatory (α_1, β) or inhibitory (α_2) effects on cell signaling, have been identified as widespread binding sites for the action of NE (Hein, 2006). Given the neuromodulatory properties of NE, the primary role of the LC-NE system is to modulate its targets in order to induce and/or maintain behavioral states and state-dependent cognitive processes (Poe et al., 2020). The LC-NE system is therefore involved in regulating a broad range of brain functions and processes (D. J. Chandler et al., 2019), including arousal, attention, autonomic activity, emotional regulation, memory, sensory processing, nociception, or stress.

The LC-NE system fulfills its functions through two modes of functioning, defined by either tonic or phasic discharge patterns (Devilbiss, 2019). During wakefulness, tonic LC-NE activity is state-dependent and covaries with arousal levels (Aston-Jones and Bloom, 1981; Aston-Jones et al., 2007). In addition to tonic discharge rates, phasic bursts of LC-NE neurons are elicited when confronted with novel or salient stimuli, and this phasic LC activity is mirrored by frequency increases in electroencephalography (EEG) and behavioral markers of attention and alertness (Holland et al., 2021). Beside electrophysiological recordings, the activity of the LC-NE system can be indirectly assessed through proxy measures derived from LC-NE neuronal activity, such as extracellular levels of NE (Mather, 2021) or variations in pupil size (Joshi and Gold, 2020).

The locus coeruleus-norepinephrine system and the anatomy of the sleep-wake circuitry

The LC-NE system is part of the reticular formation, a network of nuclei composing the ascending and descending pathways. While the latter deals with regulation of sensory and motor aspects (*e.g.* nociception, muscular tonus) and will not be considered here, the position of the LC-NE system in the former is the focus of the present section. In addition to the LC, the ascending arousal system includes the pedunculopontine (PPT) and laterodorsal tegmental (LDT) nuclei, the raphe nucleus, and the ventral tegmental area (VTA), releasing acetylcholine (Ach), serotonin (5-HT) and dopamine, respectively. Together with the basal forebrain (Ach), the hypothalamus (histamine and orexin), and the action of fast neurotransmitters (glutamate and GABA), these wakefulness-promoting systems are opposed to the sleep-promoting action of GABAergic and galaninergic neurons of the preoptic area, melanin-concentrating hormone (MCH) neurons of the hypothalamus, and

GABAergic neurons of the parafacial zone. Altogether, this intricate sleep-wake circuitry constitutes the neurobiological underpinning of sleep and wakefulness regulation (for a detailed visualization of the sleep-wake circuitry and pathways, see (Saper and Fuller, 2017)).

More than 40 years ago, retrograde tracing studies suggested that LC-NE neurons were topographically organized according to their target projection sites (Mason and Fibiger, 1979; Poe et al., 2020), and this modular architecture was later confirmed with the use of viral-genetic approaches (Schwarz et al., 2015). Within the sleep-wake neurobiological network, LC-NE neurons were found to project to cholinergic and GABAergic neurons of the basal forebrain, GABAergic neurons of the ventrolateral preoptic area (VLPO) in the anterior hypothalamus, orexinergic neurons of the lateral hypothalamus, serotoninergic neurons of the dorsal raphe, and cholinergic neurons of the PPT nucleus (Lew et al., 2021; Samuels and Szabadi, 2008; Saper and Fuller, 2017) (**Fig. 1**).

Likewise, afferent projections to LC-NE neurons were initially quantified with retrograde labeling techniques (Aston-Jones et al., 1991), and the picture was recently refined by viral tracing methods, showing that the LC receives connections from more than 100 brain regions (Schwarz et al., 2015). Among the sleep-wake circuitry, LC-NE neurons receive inputs from orexinergic neurons of the lateral hypothalamus, GABAergic neurons of the VLPO and ventral lateral hypothalamus, histaminergic neurons of the tuberomammillary nucleus (TMN), dopaminergic neurons of the VTA, serotoninergic neurons of the dorsal raphe, cholinergic neurons of the PPT and LDT, and dopaminergic neurons of the periaqueductal grey matter (Lew et al., 2021; Samuels and Szabadi, 2008; Saper and Fuller, 2017) (**Fig. 1**).

Importantly, the existence of GABAergic LC neurons located in the dendritic field surrounding the LC nucleus (termed pericerulear or peri-LC region), and also found intertwined with LC-NE neurons, was recently identified as serving an important inhibiting function to locally regulate LC-NE tonic and phasic activity (Breton-Provencher and Sur, 2019).

This complex afferent-efferent organization, complemented by a local gain mechanism based on GABAergic inhibition of LC-NE activity, allows LC-NE neurons to integrate information from multiple sources and act as a broadcasting center for the whole brain, which constitutes a crucial feature to support the many roles of the LC over multiple timescales (D. J. Chandler et al., 2019). Particularly for sleep-wake regulation, the anatomical interconnections with several sleep- and wake-promoting nuclei place the LC as an important contributor to the onset and maintenance of sleep and wakefulness states, as well as to their associated behavioral and electrophysiological properties.

The locus coeruleus-norepinephrine system and the sleep-wake cycle

Early electrophysiological, pharmacological, and lesion studies

Early electrophysiological studies on the activity of the LC-NE system across the sleep-wake cycle in rodents, cats, and monkeys, established that tonic LC activity progressively decreases when animals switch from engaged, behaviorally active states (~3Hz) to quieter,

resting conditions (~1Hz), to slow wave sleep (< 1Hz) (Chu and Bloom, 1973; Foote et al., 1980). During sleep, LC-NE neurons were found to anticipate sleep-to-wake transitions, as they display bursts of activity in the seconds preceding spontaneous or sensory-evoked awakenings (Aston-Jones and Bloom, 1981). This state-dependent LC-NE neuronal discharge pattern was further corroborated by reports showing that NE levels in the pons, amygdala, and hippocampus are high during wakefulness, lower during quiet wake, and lowest during sleep (Kalen et al., 1989; Shouse et al., 2000). Likewise, a novel experimental paradigm that allows to track pupil diameter across wakefulness and sleep states in rodents revealed that pupil size was progressively smaller when shifting from wakefulness to sleep, mirroring the gradual silencing of LC-NE neurons across behavioral states (Yüzgeç et al., 2018).

Accordingly, a series of pharmacological studies investigating the impact of modulating LC-NE activity on sleep-wake periods showed that injection of α_2-adrenergic receptor agonists into the LC area, such as clonidine or dexmedetomidine, or combined α_1- and β-adrenergic receptor blockade, suppressed LC-NE activity and induced dose-dependent sedative states in rats (Berridge and España, 2000; Correa-Sales et al., 1992; de Sarro et al., 1987). In contrast, activating LC-NE neurons through yohimbine, an α_2-adrenergic receptor antagonist, increased wakefulness (de Sarro et al., 1987).

Unlike pharmacological studies, early lesion studies in rodents, rabbits, and cats provided conflicting evidence about the consequences of LC damages on sleep-wake states, with some reporting increased wakefulness and reduced drowsiness (Braun and Pivik, 1981; Cespuglio et al., 1982) while others found acute suppressed wakefulness (Jones et al., 1973) or limited to no effect on time spent in wakefulness or sleep states (Caballero and De Andrés, 1986). Of important note, the coverage and accuracy of LC lesions in the aforementioned studies were inherently linked to the technique used (*e.g.* electrolytic-, neurotoxic-, cryo-lesion). Thus, these inconsistencies may be tied to the extent of LC injuries and to robust compensatory responses within surviving LC neurons that help sustain NE release and post-synaptic NE uptake, as long as no more than 90% of the LC is damaged (Berridge et al., 2012), a threshold that was usually not reached in those studies. More recently, specific lesioning of more than 95% of LC-NE neurons in rats did not affect the total duration of wakefulness per 24h (Blanco-Centurion et al., 2007; Gompf et al., 2010), but significantly compromised the promoting effect of exposure to a novel environment on the maintenance of wakefulness (Gompf et al., 2010).

Saper and colleagues proposed the so-called ‘flip-flop’ mechanism to describe the transitions between wakefulness and sleep (Saper et al., 2001). According to this model, two mutually inhibitory circuits are driving the onset and maintenance of wakefulness and sleep, forming a bi-stable switch which supports consolidated periods and helps preventing intermediate states: wakefulness is considered to be driven mainly by the influences of monoaminergic neurons, *i.e.* LC-NE, serotoninergic neurons from raphe nuclei, histaminergic neurons from TMN, and cholinergic neurons of PPT and LDT, while sleep is promoted through the inhibitory action on this arousal circuit by GABAergic and galaninergic neurons

located in the VLPO and median preoptic nuclei (Saper et al., 2010). Importantly, the development of novel methods covering pharmacogenetics, chemogenetics, and optogenetics, was instrumental in enabling new experimental manipulations aimed at unraveling the precise role of the LC-NE system within this framework.

Shedding new light on the Blue Spot

In 2010, Carter *et al.* demonstrated that photoinhibition of LC-NE neurons during the active period caused a reduction in time spent in wakefulness as well as an increase in wake-to-sleep transitions. Conversely, photoactivation of LC-NE neurons during the inactive period produced immediate sleep-to-wake transitions (Carter et al., 2010). Moreover, using a dual optogenetic approach, they further showed that photoactivation of orexinergic neurons of the lateral hypothalamus, which send dense projections to the LC (Horvath et al., 1999), concomitant with photoinhibition of LC-NE activity prevented sleep-to-wake transitions, while simultaneous photoactivation of both nuclei significantly increased the probability of awakenings (Carter et al., 2012). Hence, the LC-NE system also serves as a necessary and sufficient gateway for the effect of upstream arousal-regulating neuronal ensembles (Carter et al., 2013, 2012). More recently, the role of LC-NE activity in sleep-to-wake transitions was further expanded to awakenings triggered by external perturbators, and it was shown that the probability to transition from sleep to wakefulness in response to auditory stimuli was increased after photoactivation of LC-NE neurons and decreased after their photoinhibition in rats (Hayat et al., 2020). Altogether, these compelling optogenetic findings demonstrate that LC-NE activity is crucially involved in the regulation of both endogenous and sensory-evoked transitions from sleep to wake states (**Fig. 2**).

Importantly, while the LC consists mainly of NE-ergic neurons, other chemical compounds, including wake-promoting neurotransmitters such as dopamine, are produced or co-released by LC neurons (Oh et al., 2019b). A recent study therefore used optogenetics combined with LC-NE cell-type specific selective knockdown of dopamine beta hydroxylase, a necessary enzyme for NE synthesis: after this specific genetic disruption of NE production in mice, the duration of wakefulness was reduced and optogenetically-driven sleep-to-wake transitions immediately following stimulations of LC-NE neurons were abolished (Yamaguchi et al., 2018), supporting the essential role of NE for LC-mediated regulation of wakefulness periods.

Additional influences: importance of circadian and homeostatic factors

It is well established that the organization of the sleep-wake cycle is regulated by overarching circadian (‘process C’) and homeostatic (‘process S’) factors (Borbély et al., 2016), and that alteration of these two processes in the course of human aging underlie the age-related changes in sleep and wake phenotypes (Mander et al., 2017). Crucially, a series of evidence suggest that the circadian and homeostatic processes may directly or indirectly involve the LC-NE system (Aston-Jones et al., 2007).

Trans-synaptic retrograde tracing revealed that the LC receives indirect input from the suprachiasmatic nucleus (SCN) of the hypothalamus, the central pacemaker responsible for

the generation of circadian rhythms, through critical relay nuclei including the dorsomedial hypothalamic nucleus (DMH) (Aston-Jones et al., 2001). In that same study, Aston-Jones *et al.* reported that tonic LC-NE activity itself displays a certain degree of circadian variations: in rats maintained in free-running conditions under constant darkness for at least three days, LC-NE neurons were found to discharge faster during the active circadian period compared to the inactive circadian period. In addition, they showed that such circadian fluctuations in LC-NE activity was abolished after DMH lesioning (Aston-Jones et al., 2001). The existence of circadian rhythmicity within the LC-NE system was further corroborated by reports of circadian influences on NE content within the LC and SCN (Semba et al., 1984) and on the number of α- and β-receptors in the rat brain (Wirz-Justice et al., 1980), as well as circadian variations in steady-state pupil size (Daguet et al., 2019; Van Egroo et al., 2019a) and in glucose metabolism in the LC area (Buysse et al., 2004) in humans. Overall, these findings suggest that, as part of the SCN-DMH-LC circuit, the LC-NE system is under strong circadian influence and, in turn, contribute to the circadian regulation of the sleep-wake cycle (Szabadi, 2018).

With regards to homeostatic influences, the increase in slow wave sleep after sleep deprivation, which constitutes a gold standard marker of the wake-dependent build-up of sleep need, was largely dampened by lesioning the LC-NE system in rats (Cirelli et al., 2005; Cirelli and Tononi, 2004; González et al., 1996). Interestingly, release of NE during wakefulness strongly promotes synaptic potentiation (Tully and Bolshakov, 2010), which has been directly related to the amount of slow-wave activity (0.5-4Hz) during the following night (Huber et al., 2007; Vyazovskiy et al., 2009). Moreover, *in vivo* microdialysis experiments in mice showed that NE levels in the prefrontal cortex increase during prolonged wakefulness, and that LC-NE neurons projecting to the medial prefrontal cortex were particularly affected by neural fatigue (Bellesi et al., 2016), which may contribute to the specific cognitive impairment resulting from sleep deprivation (*i.e.* vigilance, working memory).

The locus coeruleus-norepinephrine system and sleep macrostructure

Akin to the discrepancy observed in LC-NE discharge rate between wakefulness and sleep, the activity of the LC-NE system is differentially regulated across sleep stages. In 1975, Hobson *et al.* described that transitions from NREM to REM sleep were anticipated by an increase in activity within a pool of cholinergic neurons located in the gigantocellular tegmental field (FTG), concomitant with a silencing of neurons from the posteroventral LC (Hobson et al., 1975). Therefore, the authors proposed a computational model revolving around a reciprocal inhibitory interplay between REM-OFF LC-NE and REM-ON cholinergic FTG neurons to regulate the onset and offset of REM episodes (McCarley and Hobson, 1975). Accordingly, early electrophysiological observations indicated that reduced, but existent tonic LC-NE activity occurred during NREM sleep, while LC-NE neurons were virtually silent during REM sleep (Aston-Jones and Bloom, 1981; Foote et al., 1980) (**Fig. 2**). Likewise, REM sleep has been associated with the lowest level of extracellular NE in the amygdala and in the pons (Shouse et al., 2000) and with the highest degree of pupil constriction (Yüzgeç et al., 2018), reflecting almost complete inactivity of the LC-NE system.

As for the investigation of the sleep-wake cycle, conflicting evidence arose from lesion studies assessing the impact of damaging the LC on NREM and REM duration (Blanco-Centurion et al., 2007; Braun and Pivik, 1981; Caballero and De Andrés, 1986). In addition, neither optogenetic inhibition nor stimulation of LC-NE neurons yielded significant changes on the duration of REM episodes or the probability to transition from NREM to REM sleep (Carter et al., 2010). These latter observations led to the hypothesis that the LC-NE system would be involved in the modulation of REM sleep rather than directly contributing to its genesis (Saper et al., 2010; Saper and Fuller, 2017); a causal role that has now been principally attributed to mutually inhibitory cell groups located in the mesopontine tegmentum: the REM-ON glutamatergic and GABAergic cells of the sublaterodorsal tegmental nucleus (also termed subcoeruleus region in humans) and the REM-OFF GABAergic neurons of the ventrolateral periaqueductal grey matter and lateral pontine tegmentum (Peever and Fuller, 2017). It is important to note, however, that the effort to identify the neurobiological circuit underlying REM sleep control is still ongoing, and the contributions of additional nuclei, are continuously being unveiled (Kroeger et al., 2019; Valencia Garcia et al., 2018).

The LC-NE system during REM sleep: a silence that speaks volume

The complete silencing of LC-NE neurons observed during REM sleep episodes is a unique condition for the brain (Van Someren, 2020). Among the many purposes attributed to REM sleep, it is therefore understandable that some of them directly relate to the (dys)function of the LC-NE system (Peever and Fuller, 2017). For instance, it was proposed that the temporary NE-free milieu inherent to REM sleep constitutes a prerequisite for the upregulation of NE receptors after the continuous exposure to NE during wakefulness and NREM sleep (Siegel and Rogawski, 1988). Others proposed that the silence of LC-NE neurons during REM sleep acts as a critical process to maintain an optimal level of brain excitability, based on a series of observations demonstrating that elevated concentrations of NE follow specific REM sleep deprivation and that the resulting excess of NE induces aberrant neuronal excitability through modulation of Na-K ATPase activity (Amar and Mallick, 2015; Khanday et al., 2016).

Importantly, the activity pattern of the LC-NE system during sleep has also been recently linked to the consolidation of memories (Poe, 2017; Sara, 2017), with a particular emphasis on the sleep-dependent processing of emotional memories (Goldstein and Walker, 2014). Within that framework, the absence of LC-NE neuronal activity during REM sleep is proposed to provide synapses with a suitable neuromodulatory environment that allows for neuronal depotentiation, which is otherwise blocked by the effect of NE, and subsequent plastic rewiring of memory schemas in the hippocampus (Poe, 2017), while the timely discharge of LC-NE neurons during NREM sleep would promote plasticity during memory replay (discussed in more details in the following section) (Sara, 2015). With regards to consolidation of emotional memories, LC-NE neuronal silence during REM episodes is thought to support the integration of the content of a given emotional event in memory networks while disconnecting and downplaying its associated arousal in limbic structures, so that the event can be subsequently recalled without triggering the original emotional reaction

(Van Someren, 2020). Particular attention has therefore been allocated towards elucidating the emotional function of LC-NE silence during REM sleep, as its disruption –or an endogenous predisposition to be disrupted– has been further postulated to strongly contribute to certain psychiatric conditions, such as post-traumatic stress disorder (PTSD) (Vanderheyden et al., 2014) and insomnia (Van Someren, 2020). Correspondingly, novel experimental findings in humans showed that the sleep-dependent adaptation to stressful stimuli or self-conscious emotions is impaired after restless REM episodes, reflecting abnormal activity of the LC-NE system during REM sleep, in healthy individuals and insomnia patients (Gong et al., 2021; Wassing et al., 2019a) as well as in PTSD (Lipinska and Thomas, 2019).

Altogether, these elements provide a strong rationale to further investigate the causes, correlates, and consequences of abnormal LC-NE activity during critical time windows of supposed silence, especially given the foreseeable clinical applications among several LC-NE-associated psychiatric and neurological disorders, but also in non-pathological aging which typically involves an increase in time spent in lighter sleep stages at the expense of REM sleep.

The locus coeruleus-norepinephrine system and sleep microstructure

While investigating LC-NE unit activity across the sleep-wake cycle, Aston-Jones & Bloom already noted that, during NREM sleep, tonic LC-NE activity displayed consistent fluctuations around spindles (trains of transient 12-16Hz waves): LC-NE neurons became almost silent in the seconds preceding the onset of a spindle, substantially increased firing during spindle activity, and decreased discharge again after the spindle offset (Aston-Jones and Bloom, 1981). These findings were recently refined and expanded by fiber photometry and optogenetic studies in rodents demonstrating that troughs in extracellular NE concentration during NREM sleep concurred with spindle activity (Kjaerby et al., 2020; Osorio-Forero et al., 2021), and that stimulation of LC-NE neurons reduced spindle occurrence (Hayat et al., 2020; Osorio-Forero et al., 2021) (**Fig. 2**) and impaired sleep-dependent memory consolidation (Novitskaya et al., 2016; Swift et al., 2018), while their inhibition increased spindle density and interfered with their temporal distribution, specifically through altered NE signaling in the thalamus (Osorio-Forero et al., 2021). In addition to the interplay with spindle activity, electrophysiological recordings in rats further showed that LC-NE neurons display a learning-dependent increase in activity during NREM sleep (Eschenko and Sara, 2008), and that this activity is phase-locked to the rising phase of NREM cortical slow oscillations (Eschenko et al., 2012) (**Fig. 2**).

Overall, these findings point at an overarching function for LC-NE neurons to provide memory circuits with an optimal neuromodulatory background; a dual task which involves balancing between promoting synaptic potentiation during NREM sleep and synaptic depotentiation during REM sleep (Van Someren, 2020). Importantly, the interplay between LC-NE activity and sleep microstructure might be particularly relevant in human aging, as the preservation of those microstructural aspects have been linked to preserved cognitive performance in older adults (Djonlagic et al., 2020).

The locus coeruleus-norepinephrine system and sleep-wake disruption in human aging and Alzheimer's disease

The vast majority of the findings described so far are based on animal studies, which provide inherent advantages when characterizing the consequences of experimental manipulation of LC-NE neurons on wakefulness and sleep. However, early evidence from human studies also contributed to identify and characterize the important role of the LC-NE system for sleep-wake regulation mechanisms. Almost 50 years ago, studies in Parkinson's disease or progressive supranuclear palsy (PSP) patients suggested that damage to the LC may underlie the observed disruption of wakefulness and sleep periods, including alteration of EEG features across sleep stages, increased nocturnal awakenings, and suppression of REM episodes (Gross et al., 1978; Mouret, 1975). A single case study in a young adult with cerebral palsy further reported that electrical stimulation of the LC led to an increase of wakefulness at the expense of REM sleep (Kaitin et al., 1986). Although limited, these initial observations hint at the translational potential of animal findings and support the relevance of investigating LC-NE-mediated sleep-wake alterations in conditions associated with LC injuries in humans. Here, we argue that Alzheimer's disease (AD) provides a valuable research perspective, given that the LC-NE system holds a crucial position by being at the intersection of initial AD-related pathophysiological processes (Braak et al., 2012) and sleep-wake dysregulation. In this final section, we will gather evidence supporting that the LC-NE system constitutes a strong neurobiological candidate underlying the sleep-wake disturbances commonly observed along the progression of AD, and we will examine emerging opportunities to test this assumption that are feasible due to recent advances in the assessment of LC-NE structural and functional properties *in vivo*.

Sleep-wake regulation and AD pathogenesis: identifying a new locus of action

As illustrated by a growing body of evidence over the past decade, sleep-wake dysregulation has emerged as a potent modifiable factor to slow down the characteristic pathophysiological processes of the disease, *i.e.* the accumulation of beta-amyloid (Aβ) and tau misfolded proteins together with neurodegeneration, as early as during the preclinical stages of the disease (Van Egroo et al., 2019c). Indeed, important discoveries established that the disruption of sleep and wakefulness constitutes a core mechanism of early AD pathogenesis: the physiological dynamics of both Aβ and tau proteins, encompassing their release and clearance are regulated by the sleep-wake cycle (J. K. Holth et al., 2019; Xie et al., 2013; Yamada et al., 2014). Conversely, both Aβ and tau pathology *per se* induce alteration of the sleep-wake cycle in AD mouse models (Holth et al., 2017; Jee Hoon Roh et al., 2012), supporting bidirectionality in the relationships between sleep-wake disturbances and AD-related neuropathological processes (Van Egroo et al., 2019c).

Crucially, landmark *post-mortem* studies in humans revealed that, beside its close connection with sleep-wake regulation described throughout this review, the LC is among the first sites of tau pathology across the lifespan (Braak et al., 2012), such that the vast majority of individuals harbor abnormally phosphorylated tau in the LC by the age of 40 (Braak et al., 2011a). In addition, the consequences of accumulated tau burden within the LC were found

to be specifically expressed in AD cases compared to other tauopathies (*i.e.* PSP, cortico-basal degeneration), as substantial LC neuronal loss appeared as a phenotype exclusive to AD (Oh et al., 2019a).

Combined, these observations lend support to a theoretical framework in which the LC-NE system would constitute a bridge connecting sleep-wake dysregulation and initial AD-related pathophysiological processes (**Fig. 3**). In mice, chronic sleep disruption or intermittent short sleep for three days a week during one month in mice was sufficient to produce profound alterations in LC-NE morphology, as evidenced by a drastic reduction of LC-NE neuronal counts and axonal projections (Zhu et al., 2016). Moreover, in a mouse model of tauopathy, repeated exposures to shortened sleep accelerated tau accumulation within LC-NE neurons and its progression to the entorhinal cortex, hippocampus, and amygdala, and advanced the onset of neurobehavioral deficits (Zhu et al., 2018). Remarkably, these effects were long-lasting, with structural alteration of the LC-NE system and cognitive impairments persisting one year after the chronic sleep disruption protocol (Owen et al., 2021). These animal findings suggest that the negative consequences of early-in-life sleep-wake dysregulation precipitate and sustain AD-related processes within the LC-NE system.

Bringing similar research questions into human studies has long been hindered by the considerable challenge to image the LC-NE, due to its deep location and its small size. However, recent advances in MRI methods now allow to investigate LC-NE structural and functional integrity with relatively short acquisition times to accommodate clinical studies (Kelberman et al., 2020). Importantly, the benefits of such sequences are exponentially amplified when implemented at ultra-high field (*i.e.* ≥ 7 Tesla MRI), given the sub-millimeter resolution available to identify and characterize the LC (Priovoulos et al., 2018). In that context, it was established that MRI-assessed LC-related contrast, supposedly reflecting neuronal and fiber projection density (Priovoulos et al., 2020), starts declining slowly in the 5^{th} decade of life in cognitively unimpaired older individuals (Liu et al., 2017), while it is markedly reduced and correlated with Aβ pathology in AD patients (Betts et al., 2019) and with tau pathology in asymptomatic older individuals (Jacobs et al., 2021).

Interestingly, a parallel may be drawn between deterioration of the LC-NE system and the unfolding of sleep-wake disruption in late life; a relationship that appears even more striking during the course of AD. Sleep disturbances are common in AD patients, starting as early as in the preclinical stages under the form of exacerbated age-related sleep-wake disruption (Van Egroo et al., 2019c), but their prevalence and magnitude strongly increase with disease severity (Gagnon et al., 2019) which often precipitate institutionalization (Peter-Derex et al., 2015). Of note, most of these disturbances pertain to sleep-wake dimensions that have been described so far as tightly linked to the function of LC-NE system based on evidence from animal models: fragmented sleep with more frequent nocturnal arousals and awakenings, insomnia, poorer REM sleep integrity, and reduction in spindle density and slow-wave activity (Gagnon et al., 2019). Yet, while the significant degeneration of the LC-NE system observed at histological investigation of AD brains was suggested to contribute to the disruption of the sleep-wake cycle commonly experienced by AD patients (Oh et al., 2019a),

no direct assessments of sleep-wake measures were available in these *post-mortem* studies. Similarly, except for two recent studies discussed below, no *in vivo* studies correlated LC-NE properties with sleep-wake measurements in healthy aging or AD, leaving important gaps in the understanding of the interplay between alteration of the LC-NE system, sleep-wake dysregulation, and AD-related pathophysiological processes in humans. Hereafter, we propose research perspectives to address these unanswered questions, and that would pinpoint the LC-NE system as a new target connecting early sleep-wake disruption to AD pathogenesis.

Emerging opportunities and perspectives

Before addressing the context of AD, an important first step for the field will be to characterize the relationships between LC-NE structural/functional properties and sleep-wake regulation across the human lifespan. Experimental protocols should therefore take advantage of the aforementioned MRI methods, ideally at ultra-high field, to establish the links between fine-grained LC-related MRI metrics and subjective (e.g. sleep questionnaires) as well as objective (e.g. EEG, actigraphy) sleep-wake dimensions in different age groups. For example, aligning with previous work in animals, it would be informative to assess whether the structural integrity of the LC-NE system or its resting-state functional activity patterns (Gong et al., 2021; Jacobs et al., 2020), is associated with the relative duration or distribution of EEG-derived sleep stages in humans. For that purpose, particular attention should be allocated to examine non-linear associations (Jacobs et al., 2018; Liu et al., 2019) as well as the influence of confounding factors such as depression or anxiety levels, which are directly related to both the LC-NE system (Grueschow et al., 2021) and sleep-wake quality (Van Someren, 2020). In addition, the inherent difficulty to record human sleep in MRI, *a fortiori* in older individuals, may tend to force comparison of EEG-derived sleep features acquired outside the scanner with LC functional characteristics measured during wakefulness. Existing studies using simultaneous EEG/fMRI during sleep usually collect only incomplete sleep cycles during which naturally occurring REM sleep is not reached while inside the scanner.

In the context of AD, additional animal work relating glymphatic clearance of toxic proteins to fluctuations of LC-NE activity across the sleep-wake cycle would provide valuable insights into the potential pathways linking the LC-NE system to AD pathogenesis through sleep-wake dysregulation. Indeed, in their landmark work on the role of the sleep-wake cycle for metabolite clearance in mice, Xie *et al.* demonstrated that elevated NE levels impaired glymphatic function by restricting the interstitial space volume (Xie et al., 2013), but no direct assessment of LC-NE activity was available in that study. In turn, existing optogenetic studies modulating LC-NE activity across the sleep-wake cycle have not evaluated its impact on glymphatic clearance, nor did they investigate the long-term effect of chronic LC-NE photoactivation/photoinhibition on Aβ or tau protein burden in AD mouse models. In humans, clear-cut evidence and methods to measure such a sleep-dependent glymphatic clearance system are still lacking (Mestre et al., 2020), although one recent study by Fultz *et al.* provides support to the existence of a temporally ordered sequence of events during sleep in

the human brain, linking the occurrence of slow waves to CSF flow through hemodynamic oscillations (Fultz et al., 2019).

Importantly, the relationships between alteration of the LC-NE system and sleep-wake disturbances should be investigated across the different stages of the disease, and their combined contribution to forecast cognitive trajectories should be evaluated. One recent study by Elman *et al.* provides preliminary evidence in that direction, indicating that reduced MRI-assessed LC-NE contrast is associated with worse self-reported metrics of daytime functioning among 481 older men from the Vietnam Era Twin Study of Aging, and that both lower LC-NE structural integrity and increased daytime dysfunction act as independent predictors of increased odds of conversion to mild cognitive impairment (Elman et al., 2021). We showed that lower integrity of the middle-to-caudal LC, as measured with a 7T LC-NE MRI sequence, is associated with more frequent self-reported nocturnal awakenings in 72 cognitively unimpaired older adults, particularly in individuals with higher plasma levels of total tau protein burden (Van Egroo et al., 2021b), a biomarker of increased risk for cognitive decline and incident dementia. Additional longitudinal designs with repeated cognitive, sleep-wake, and LC MRI assessments in cognitively impaired and unimpaired older individuals will be crucial to shed light on the temporal ordering of events, and will contribute to understanding the isolated protective effects of preserved sleep-wake regulation (Shi et al., 2018) and LC-NE integrity (Mather and Harley, 2016b) for cognitive performance in aging and AD.

Of note, the specificity of the LC-NE system in the aforementioned relationships will have to be tested against the isolated role of other wake-promoting neuronal populations that also display marked neurodegeneration in AD, such as the orexinergic cells of the lateral hypothalamus or the histaminergic neurons from the TMN with (Berteotti et al., 2021; Oh et al., 2019a). In particular, as the LC-NE system has been suggested to act as an effector of the wake-promoting inputs from the orexinergic neurons (Carter et al., 2012), the structural and functional connections between the LC and these hypothalamic neurons warrant further attention to disentangle their respective contributions to age- and AD-related sleep-wake disruption (Giorgi et al., 2021).

Finally, interventional studies interfering with sleep-wake regulation and/or modulation of LC-NE activity through pharmacological means or non-invasive stimulation techniques will be critical to address causality in the aforementioned relationships. Indirectly bolstering LC-NE function with transcutaneous vagus nerve stimulation has been proposed to harbor some positive effects on subjective sleep quality in one study of older individuals (Bretherton et al., 2019), but objective evaluation of its quantitative impact and its long-term effects on the sleep-wake cycle, potentially in combination with other sleep-wake interventions such as sleep hygiene education or cognitive behavioral therapy for insomnia, remains to be performed. In turn, the deleterious effect of fragmented sleep-wake periods on the structural integrity of the LC-NE system proposed in animal studies (Owen et al., 2021; Zhu et al., 2018) remain to be established in humans. Studies including clinical sleep interventions in populations with different degrees of sleep-wake fragmentation, such as elderly individuals,

frequent nappers, or sleep apnea patients, would thus provide valuable knowledge for further development and evaluation of preventive interventions in populations at higher risk of cognitive decline and AD trajectories.

Practice Points

1. The brainstem locus coeruleus constitutes the primary source of norepinephrine for the central nervous system, and is an important arousal-promoting nucleus of the sleep-wake circuitry.
2. Locus coeruleus-norepinephrine tonic firing rate fluctuates across the sleep-wake cycle: highest during wakefulness, lower during non-rapid eye movement sleep, and virtually silent during rapid eye movement sleep; locus-coeruleus norepinephrine activity is also influenced by both homeostatic and circadian factors.
3. During sleep, locus coeruleus-norepinephrine activity regulates sleep-to-wake transitions and is phase-locked to sleep microstructural elements such as spindles and slow waves; its silence during rapid eye movement sleep appears critical for neuronal depotentiation, brain excitability, and emotional regulation.
4. The locus coeruleus-norepinephrine system holds a crucial position at the intersection between initial Alzheimer's disease-related pathophysiological processes and sleep-wake regulation in human aging.
5. Preliminary findings based on recent MRI developments bring support to *in vivo* investigations of locus coeruleus-norepinephrine-mediated sleep-wake dysregulation in Alzheimer's disease pathogenesis.

Research Agenda

1. Quantitative *in vivo* studies in humans to better characterize the associations between locus coeruleus-norepinephrine structural/functional properties and sleep-wake regulation across the lifespan.
2. Animal studies aiming to clarify the links between glymphatic clearance of toxic proteins and locus coeruleus-norepinephrine activity across the sleep-wake cycle.
3. Longitudinal studies in individuals across the Alzheimer's disease continuum to evaluate the contribution of locus coeruleus-norepinephrine-mediated sleep-wake alterations to neuropathophysiological and cognitive trajectories.
4. Interventional studies, in animal models and in humans, aiming at modulating locus coeruleus-norepinephrine activity to address causality in the aforementioned relationships and to inform on novel preventive strategies among clinical sleep populations.

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Appendix 2

Supplemental figures and tables for the paper presented in Chapter 3.

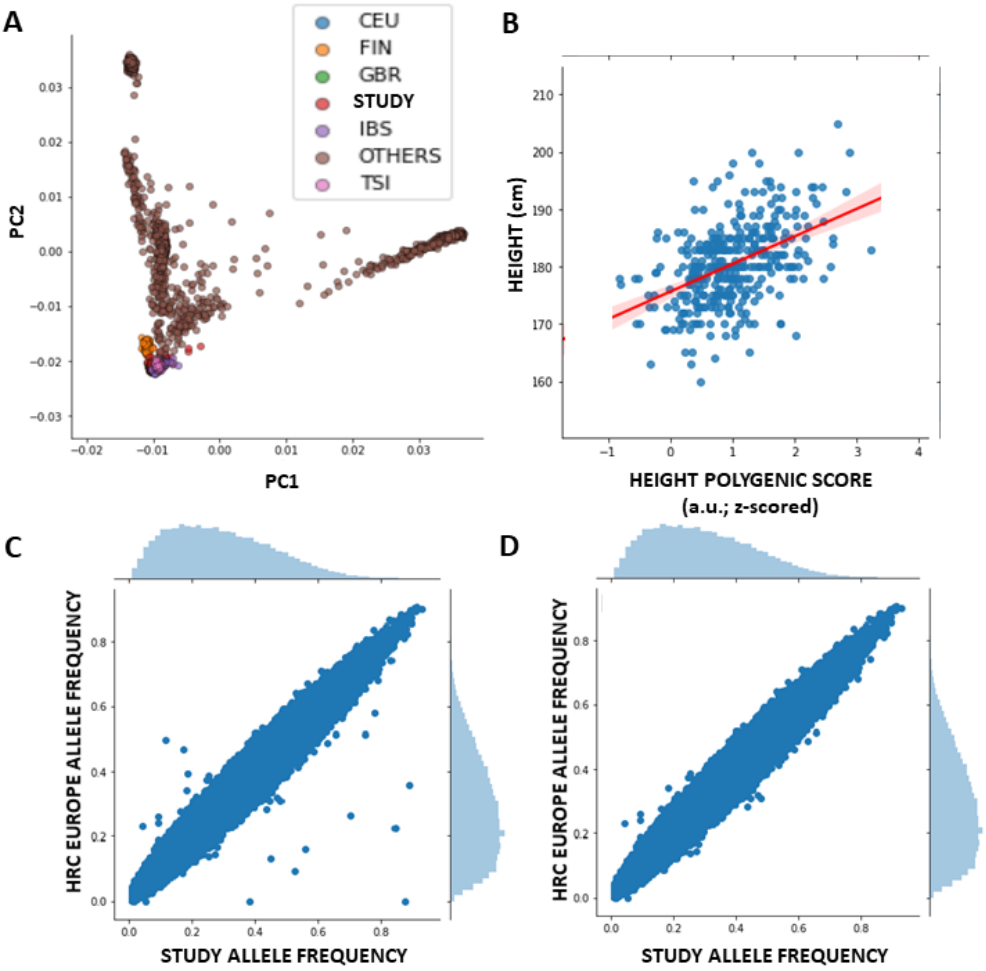

Figure 3.S1: quality checks (QC) performed during genetic data processing
A. Principal component (PC) analysis of our data (after merging with genome 1k). PC1 vs. PC2 is displayed for our European (Caucasian) study sample (STUDY) and for other ethnicities provided by Haplotype Reference Consortium (HRC) Europe release 1.1. CEU: Utah Residents (CEPH) with Northern and Western European Ancestry; FIN: Finnish in Finland; GBR: British in England and Scotland; IBS: Iberia in Spain; TSI: Toscani in Italia. Our study sample clusters at the same position as Europeans.
B. To validate common SNP assessments in our sample, we predicted height of our volunteers based on a meta-analysis of height GWAS studies [75]. Predicted Liability for Height was significantly associated with the actual values in our sample ($r = 0.46$; $p < 10^{-20}$) with similar value as reported in Yengo et al. (2018) ($r = 0.49$).
C-D. Allele frequencies in our sample were compared to HRC Europe reference data and SNP deviating more than 0.2 unit from European allele frequency were excluded [**C.** Original data. **D.** Data after removal of deviant allele].

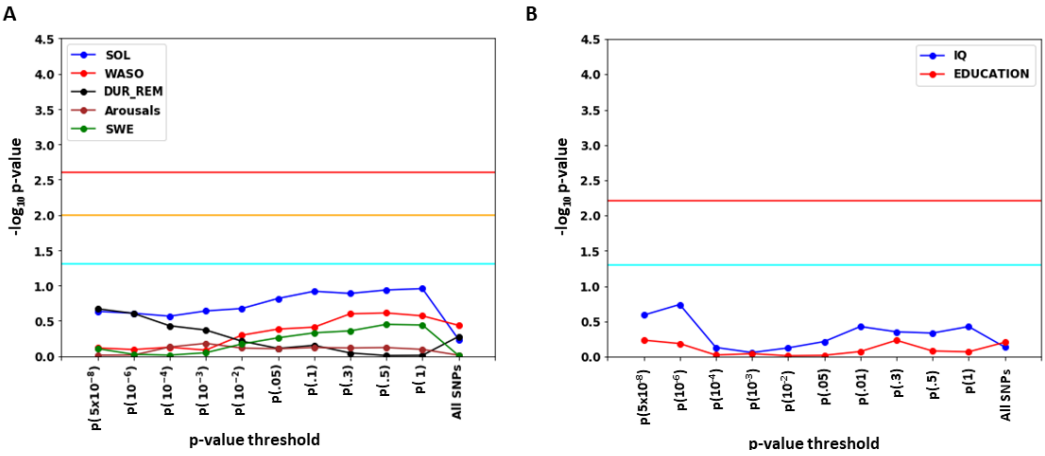

Figure 3.S2: Negative controls analysis and correlation between AD PRS and IQ or education

A. Statistical outcomes of GLMs with five sleep metrics of interest vs. polygenic prediction of individual height from conservative ($p < 5x10^{-8}$) p-value threshold to using all SNPs. Negative log transformation of p-values of the associations are presented on the vertical axis. Horizontal lines indicated different p-values thresholds: light blue = .05 (uncorrected); orange= .01 (corrected for 5 sleep metrics); red = 0.0025 (experiment-wise correction). As expected height prediction is not associated with any of the sleep metrics further validating our main finding.

B. Statistical outcomes of GLMs with IQ (N=347) and number of years of education (N=300) vs. PRS for AD from conservative ($p < 5x10^{-8}$) p-value threshold to using all SNPs. Negative log transformation of p-values of the associations are presented on the vertical axis. Horizontal lines indicated different p-value thresholds: light blue = .05 (uncorrected); red = 0.00625 (correction for 8 independent PRS). As expected IQ and education are not associated with PRS for AD.

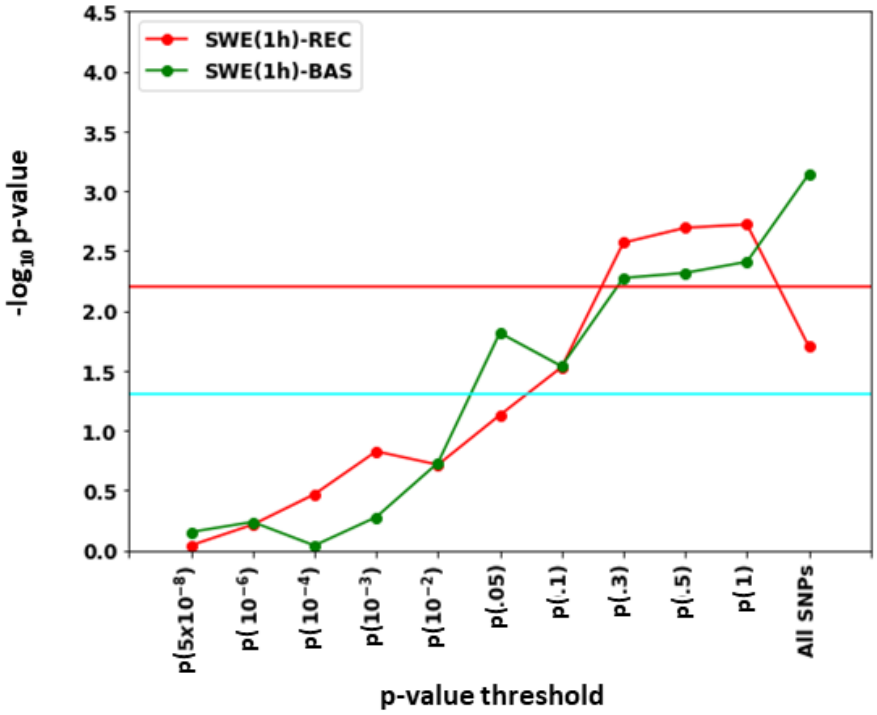

Figure 3.S3: Associations between Polygenic Risk Score (PRS) for AD and SWE in the first hour of sleep for baseline (BAS), recovery (REC) and extension (EXT) nights. Statistical outcomes of GLMs with SWE (0.5-4Hz) of each night type in the first hour of sleep (SWE(1h)) vs. AD PRS from conservative ($p < 5x10^{-8}$) p-value threshold to using all SNPs. GLMs are corrected for age and BMI. Negative log transformation of p-values of the associations are presented on the vertical axis. Horizontal lines indicate different p-values thresholds: light blue = .05 (uncorrected); red = 0.00625 (experiment-wise correction; see methods).

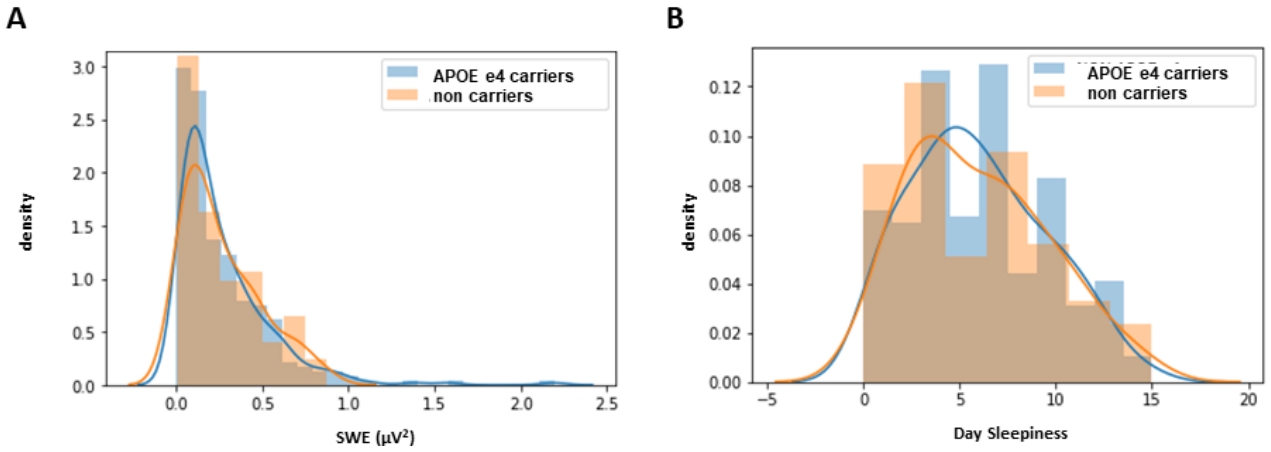

Figure 3.S4: No difference in SWE and daytime sleepiness between APOE e4 carriers and non-carriers.
Distribution of APOE e4 Carriers and Non Carriers for SWE during baseline night (**A**) and daytime sleepiness (**B**). T-tests between APOE e4 carriers (N = 100) and Non carriers (N = 263) show no significant difference in case of SWE (p = 0.84) and daytime sleepiness (p = 0.94).

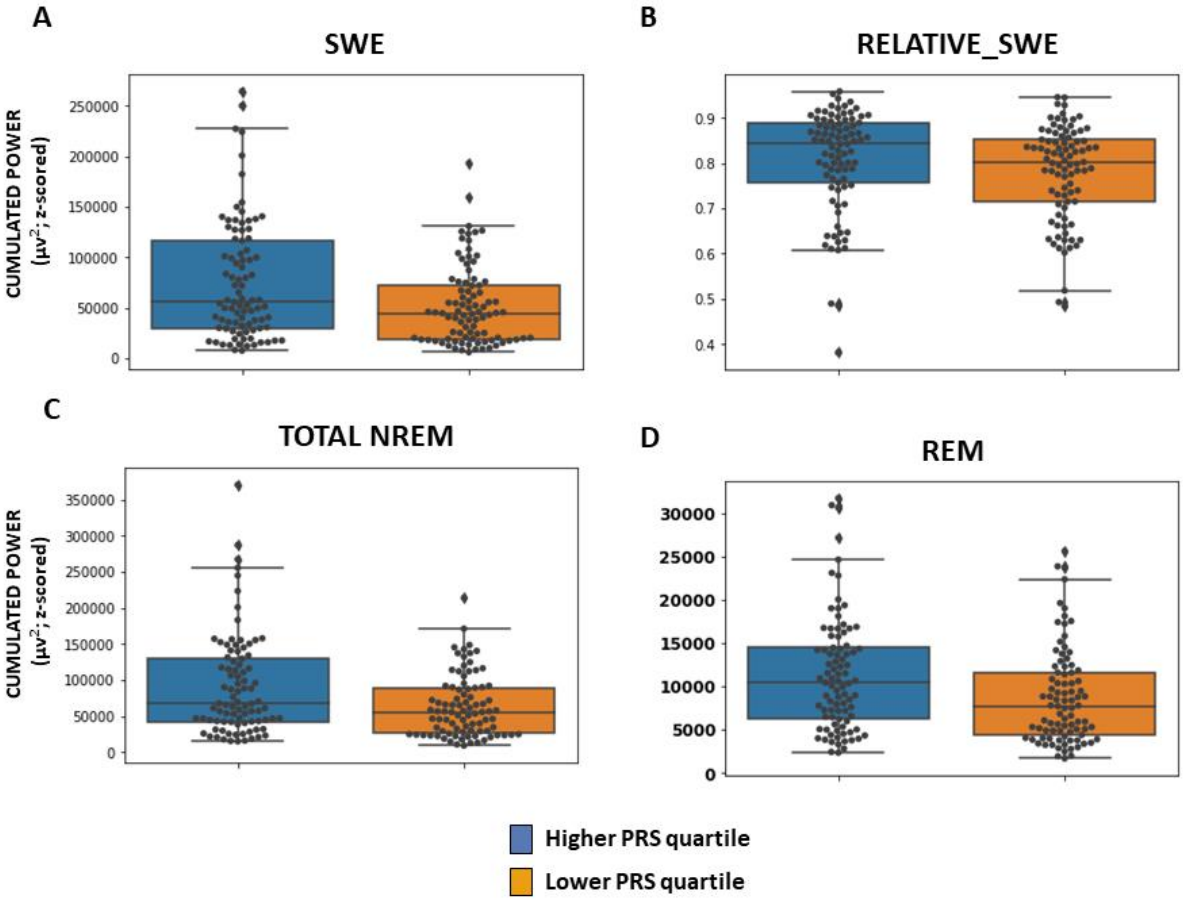

Figure 3.S5: Differences in SWE, relative_SWE, total NREM power and REM power during baseline sleep between lower and upper AD PRS quartiles.
Comparison of individual with 25% lowest (N = 90) and 25% highest (N = 90) PRS (using all SNPs) for SWE (**A**), relative SWE (**B**), cumulated total power between 0.5 and 25 Hz during NREM sleep (**C**) and cumulated power over the 2 to 6 Hz band during REM sleep (**D**). T-tests indicated higher power in higher quartile vs. lower quartile in all three absolute measures (**SWE: p = 0.008; total NREM: p = 0.008; REM: p = 0.01**) but not for **relative SWE (p = 0.14)**.
Relative power was computed as the individual ratio between SWE and cumulated total power between 0.5 and 25 Hz during NREM sleep.
Dots: individual values; boxes: interquartile interval; horizontal bar within boxes: median;
error bars: 10 to 90% interval.

Table 3.S1. Number of SNPs included in PRS computation as a function of p-value thresholding in reference GWAS summary statistic of [21].

P-value threshold	Number of SNPs included in PRS computation
P-value threshold 5 x 10^{-8} (and LD)	64
P-value threshold 10^{-6} (and LD)	108
P-value threshold 10^{-4} (and LD)	517
P-value threshold 10^{-3} (and LD)	2543
P-value threshold 0.01 (and LD)	15654
P-value threshold 0.05 (and LD)	58096
P-value threshold 0.1 (and LD)	101336
P-value threshold 0.3 (and LD)	233635
P-value threshold 0.5 (and LD)	329341
P-value threshold 1 (and LD)	466829
All SNPs (no LD)	7780254

LD: linkage disequilibrium pruning

Table 3.S2. Statistical outcomes of GLMs with the five baseline night sleep metrics of interest vs. AD PRS from conservative ($p < 5x10^{-8}$) to inclusive ($p < 1$) p-value threshold and selecting all SNPs.

p-value threshold	*p=5 10^{-8}*	*p =10^{-6}*	*p =10^{-4}*	*p=.001*	*p=.01*	*p=.05*	*p=.1*	*p=.3*	*p=.5*	*p=1*	*All SNPs*
SOL (N = 356)	ns							$\beta = -0.11$ $p = 0.03$ Age $\beta = 0.13$ $p = .01$ TST $\beta = 0.13$ $p = 0.01$ BMI $\beta = -0.19$ $p < .0003$	$\beta = -0.11$ $p = 0.03$ Age $\beta = 0.13$ $p = .01$ TST $\beta = 0.13$ $p = 0.01$ BMI $\beta = -0.19$ $p = .0003$	$\beta = -0.11$ $p = 0.04$ Age $\beta = 0.13$ $p = .01$ TST $\beta = 0.13$ $p = 0.01$ BMI $\beta = -0.19$ $p = .0003$	ns
WASO (N = 356)	ns										
DUR_REM (N = 356)	ns			$\beta = 0.1$ $p = 0.03$ Age $\beta = 0.04$ $p = .04$ TST $\beta = 0.5$ $p =< 0.0001$	ns						
Arousals (N = 356)	ns										
SWE (N = 356)	ns					$\beta = 0.12$ $p = 0.019$ Age $\beta = -0.2$ $p = .0001$ TST $\beta = -0.1$ $p = 0.04$	$\beta = 0.12$ $p = 0.018$ Age $\beta = -0.21$ $p < .0001$ TST $\beta = -0.11$ $p = 0.04$	$\beta = 0.14$ $p = 0.007$ Age $\beta = -0.2$ $p = .0001$ TST $\beta = -0.1$ $p = 0.04$	$\beta = 0.14$ $p = 0.006$ Age $\beta = -0.21$ $p < .0001$ TST $\beta = -0.1$ $p = 0.05$	$\beta = 0.15$ $p = 0.005$ Age $\beta = -0.21$ $p < .0001$ TST $\beta = -0.1$ $p = 0.05$	$\boldsymbol{\beta = 0.17}$ $\boldsymbol{p = 0.0011}$* Age $\beta = -0.22$ $p < .0001$ TST $\beta = -0.11$ $p = 0.03$

GLMs included age, BMI and TST. Only uncorrected significant association p<.05 are reported. Partial effect sizes are provided for each significant association. ns: non-significant (p > 0.05). ***** association meeting study-wise correction for multiple comparison (p < 0.00125). SOL: sleep onset latency; WASO: wake time after sleep onset; DUR_REM: duration of REM sleep; arousal: hourly rate of micro-arousal during sleep; SWE: slow wave energy in NREM sleep (0.5-4Hz). The number of participants included in each GLM, following outlier removal and because of missing data, is reported below each dependent variable.

Table 3.S3. Statistical outcomes of GLMs with SWE during the first hour of sleep (SWE 1h) in baseline (BAS) nights of sleep and when separating slow oscillation SWE (SO-SWE) and fast oscillation SWE (FO-SWE) from conservative ($p < 5x10^{-8}$) to inclusive ($p < 1$) p-value threshold and when selecting all SNPs.

p-value threshold	*p=5 10^{-8}*	*p =10^{-6}*	*p =10^{-4}*	*p=.001*	*p=.01*	*p=.05*	*p=.1*	*p=.3*	*p=.5*	*p=1*	*All SNPs*
SWE 1h BAS (N = 355)	ns					$\beta = 0.12$ $p = 0.02$ Age $\beta = -0.2$ $p = .0001$ TST $\beta = -0.17$ $p = 0.001$	$\beta = 0.12$ $p = 0.03$ Age $\beta = -0.21$ $p < .0001$ TST $\beta = -0.17$ $p = 0.001$	$\beta = 0.14$ $p = 0.005$ Age $\beta = -0.20$ $p < .0001$ TST $\beta = -0.17$ $p = 0.001$	$\beta = 0.14$ $p = 0.005$ Age $\beta = -0.21$ $p < .0001$ TST $\beta = -0.17$ $p = 0.001$	$\beta = 0.15$ $p = 0.004$ Age $\beta = -0.21$ $p < .0001$ TST $\beta = -0.17$ $p = 0.001$	$\beta = 0.17$ $p = 0.0007$ Age $\beta = -0.22$ $p < .0001$ TST $\beta = -0.17$ $p = 0.0007$
SO-SWE (N = 357)	ns					$\beta = 0.11$ $p = 0.04$ Age $\beta = -0.2$ $p = .0002$ TST $\beta = -0.14$ $p = 0.007$	$\beta = 0.12$ $p = 0.02$ Age $\beta = -0.2$ $p = .0001$ TST $\beta = -0.14$ $p = 0.007$	$\beta = 0.12$ $p = 0.02$ Age $\beta = -0.2$ $p = .0001$ TST $\beta = -0.14$ $p = 0.007$	$\beta = 0.12$ $p = 0.02$ Age $\beta = -0.2$ $p = .0001$ TST $\beta = -0.14$ $p = 0.007$	$\beta = 0.12$ $p = 0.2$ Age $\beta = -0.2$ $p = .0001$ TST $\beta = -0.14$ $p = 0.007$	$\beta = 0.16$ $p = 0.003$ Age $\beta = -0.22$ $p < .0001$ TST $\beta = -0.14$ $p = 0.007$
FO-SWE (N = 357)	ns					$\beta = 0.11$ $p = 0.03$ Age $\beta = -0.2$ $p = .0002$ TST $\beta = -0.14$ $p = 0.007$	$\beta = 0.11$ $p = 0.04$ Age $\beta = -0.2$ $p = .0001$ TST $\beta = -0.14$ $p = 0.007$	$\beta = 0.12$ $p = 0.02$ Age $\beta = -0.2$ $p = .0001$ TST $\beta = -0.14$ $p = 0.007$	$\beta = 0.12$ $p = 0.02$ Age $\beta = -0.2$ $p = .0001$ TST $\beta = -0.14$ $p = 0.007$	$\beta = 0.13$ $p = 0.01$ Age $\beta = -0.2$ $p = .0001$ TST $\beta = -0.14$ $p = 0.007$	$\beta = 0.15$ $p = 0.005$ Age $\beta = -0.21$ $p < .0001$ TST $\beta = -0.14$ $p = 0.007$

GLMs included age and BMI (and TST for SO-SWE and FO-SWE). Only uncorrected significant association p<.05 are reported. Partial effect sizes are provided for each significant association. ns: non-significant (p > 0.05). BMI was never significantly associated with AD PRS. SO-SWE: 0.5-1Hz; FO-SWE: 1.25-4Hz.

Table 3.S4. Statistical outcomes of GLMs with SWE during recovery and extension and with SWE rebound vs. AD PRS from conservative ($p < 5x10^{-8}$) to inclusive ($p < 1$) p-value threshold and selecting all SNPs.

P-value threshold	*p=5 10^{-8}*	*p =10^{-6}*	*p =10^{-4}*	*p=.001*	*p=.01*	*p=.05*	*p=.1*	*p=.3*	*p=.5*	*p=1*	*All SNPs*
SWE – REC (N = 353)	ns						$\beta = 0.11$ $p = 0.04$ Age $\beta = -0.28$ $p < .0001$	$\beta = 0.13$ $p = 0.008$ Age $\beta = -0.28$ $p < .0001$	$\beta = 0.13$ $p = 0.008$ Age $\beta = -0.29$ $p < .0001$	$\beta = 0.14$ $\boldsymbol{p = 0.006}$* Age $\beta = -0.29$ $p.0001$	$\beta = 0.11$ $p = 0.04$ Age $\beta = -0.3$ $p < .0001$
SWE 1h REC (N = 355)	ns						$\beta = 0.11$ $p = 0.03$ Age $\beta = -0.27$ $p < .0001$	$\beta = 0.16$ $p = 0.002$ Age $\beta = -0.27$ $p < .0001$	$\beta = 0.16$ $p = 0.002$ Age $\beta = -0.27$ $p < .0001$	$\beta = 0.16$ $p = 0.002$ Age $\beta = -0.27$ $p < .0001$	$\beta = 0.12$ $p = 0.02$ Age $\beta = -0.28$ $p < .0001$
SWE – Rebound (N = 344)	ns										$\beta = -0.11$ $p = 0.047$
SWE – EXT (N = 356)	ns										

GLMs included age, BMI and total sleep time (TST). Only uncorrected significant association p<0.05 are reported (in bold). ***** association meeting study-wise correction for multiple comparison ($p < 0.00625$). SWE rebound consist in the difference between difference in the SWE in the first hour of sleep of recovery and baseline nights. Partial effect sizes are provide for each significant association. ns: non-significant ($p > 0.05$). The number of participants included in each GLM, following outlier removal and because of missing data, is reported below each dependent variable.

Table 3.S5. Statistical outcomes of GLMs with non-EEG sleep metrics vs. AD PRS from conservative ($p < 5x10^{-8}$) to inclusive ($p < 1$) p-value threshold and selecting all SNPs.

p-value threshold	*p=5 10^{-8}*	*p =10^{-6}*	*p =10^{-4}*	*p=.001*	*p=.01*	*p=.05*	*p=.1*	*p=.3*	*p=.5*	*p=1*	*All SNPs*
kRA (N = 361)	$\beta = -0.11$ $p = 0.036$	$\beta = -0.13$ $p = 0.016$	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sleep-qual (N = 363)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Day-sleepiness (N = 363)	ns	ns	$\beta = 0.11$ $p = 0.043$	$\beta = 0.12$ $p = 0.02$	$\beta = 0.13$ $p = 0.01$	$\boldsymbol{\beta = 0.17}$ $\boldsymbol{p = 0.001}*$	$\beta = 0.16$ $p = 0.003$	$\boldsymbol{\beta = 0.16}$ $\boldsymbol{p = 0.002}*$	$\beta = 0.16$ $p = 0.003$	$\beta = 0.15$ $p = 0.006$	ns

GLMs included age and BMI. Only uncorrected significant association p<.05 are reported (in bold). Partial effect sizes are provided for each significant association. ns: non-significant (p > 0.05) BMI and age were not significantly associated with the dependent variables. ***** associations meeting study-wise correction for multiple comparison (p < 0.002). The number of participants included in each GLM, following outlier removal and because of missing data, is reported below each dependent variable

Appendix 3

Supplemental figures and tables for the paper presented in Chapter 4.

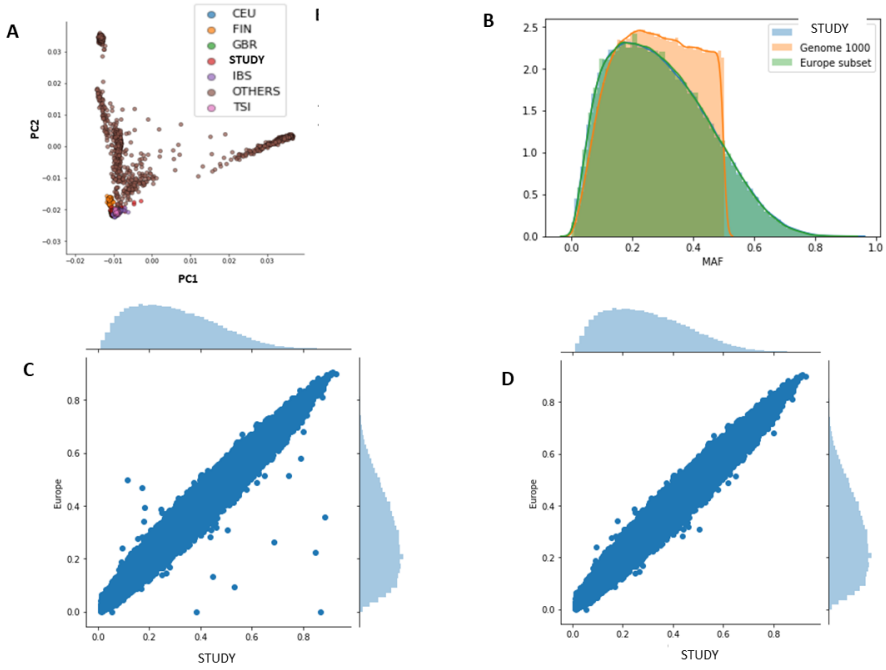

Figure 4.S1: quality checks (QC) performed during genetic data processing

A. Principal component (PC) analysis of our data (after merging with **1000 Genomes Project (**1KGP) (Auton et al., 2015)**.** Principal Component1 (PC1) vs. PC2 is displayed for our European (Caucasian) study sample (STUDY) and for other ethnicities provided by Haplotype Reference Consortium (HRC) Europe release 1.1. **CEU**: Utah Residents (CEPH) with Northern and Western European Ancestry; **FIN**: Finnish in Finland; **GBR**: British in England and Scotland; **IBS**: Iberia in Spain; **TSI**: Toscani in Italia. Our study sample clusters at the same position as Europeans.
B. Allele frequencies distribution in the 1KGP, European subset of 1KGP and our cohort (STUDY).
C-D. Allele frequencies in our sample were compared to HRC Europe reference data and SNP deviating more than 0.2 unit from European allele frequency were excluded [**C.** Original data. **D.** Data after removal of deviant alleles].

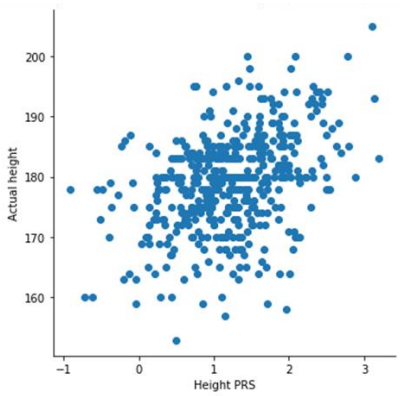

Figure 4.S2: PRS for height correlation with actual height.

To validate common SNP assessments in our sample, we computed PRS (arbitrary units, z-scored) for height of the participants based on the summary statistics for height which includes 3,290 genome-wide significant loci. PRS for height was significantly associated with the actual height values in our sample (Pearson's r = 0.4; p = $1.5*10^{-18}$) with similar value as reported in (Yengo et al., 2018): r = 0.49.

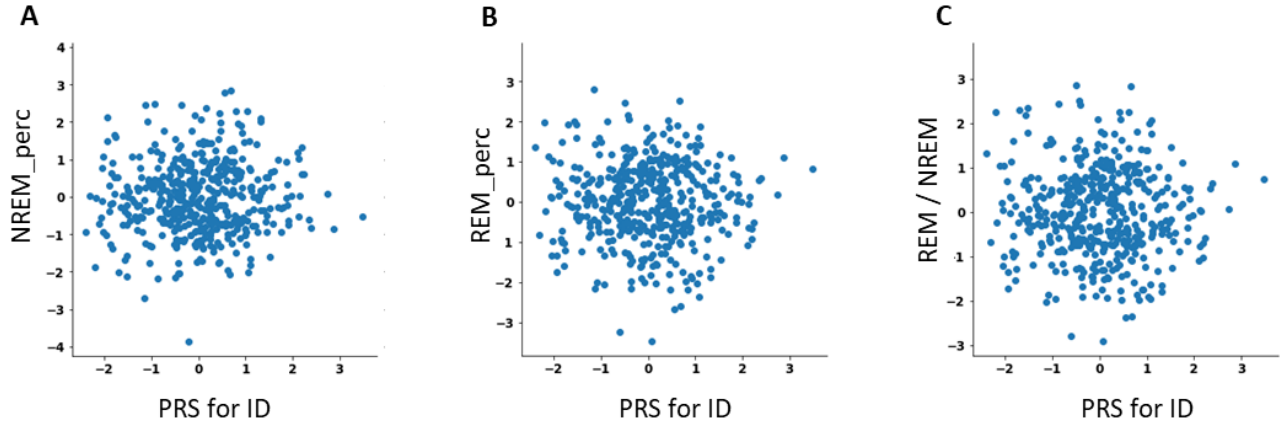

Figure 4.S3. Associations between PRS for ID and percentage of sleep stages durations.

GLM non-significant associations between PRS for ID and **(A) NREM_perc**, duration of NREM sleep as percentage of total sleep time (N = 456; GLM: β=-0.06, p=0.2;Spearman r=0.05 , p=0.29), **(B) REM_perc**, duration of REM sleep as percentage of total sleep time (N = 456; GLM: β=-0.04, p=0.5; Spearman r=-0.03 , p=0.6), **(C) REM / NREM**, ratio between REM and NREM sleep durations (N = 456; GLM : β=-0.04, p=0.35; Spearman r=-0.03 , p=0.5). . PRS for ID was computed including SNPs with p-value threshold = 1. Spearman's correlations r are reported for completeness and do not replace GLM results. All metrics on the graphs including ID PRS (arbitrary units) are z-transformed before plotting.

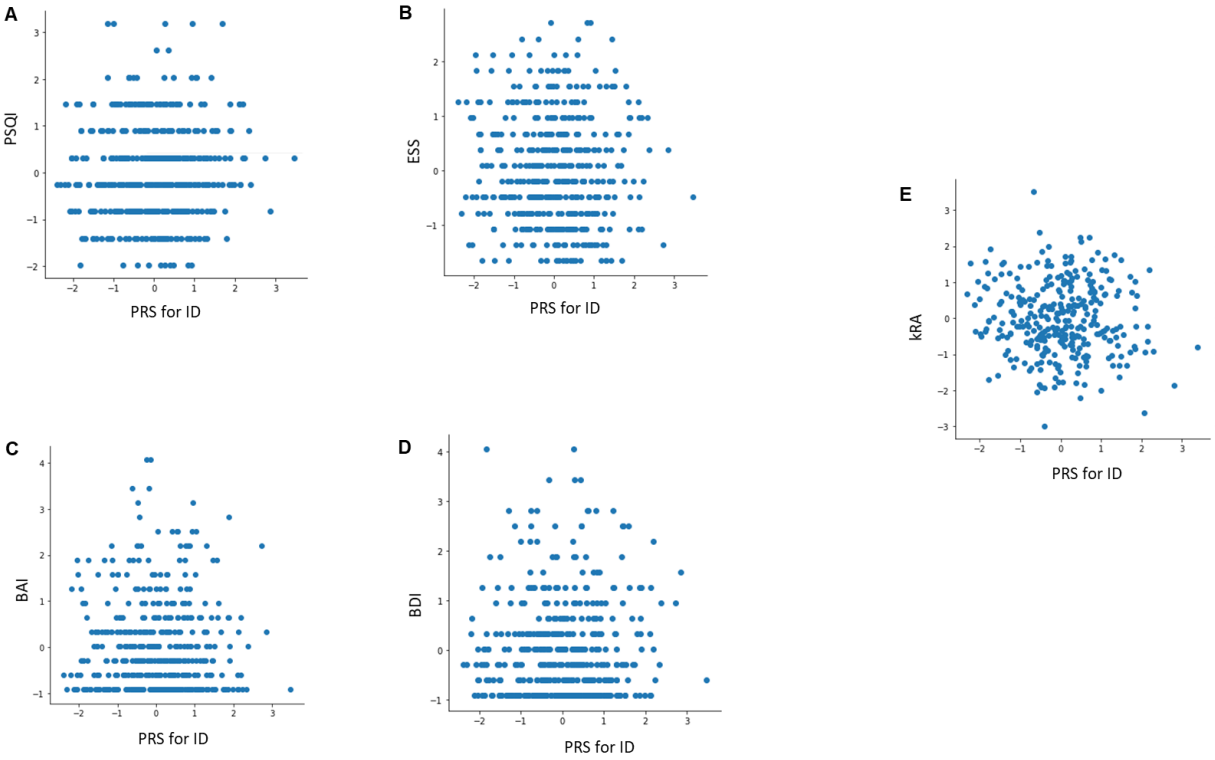

Figure 4.S4. Associations between PRS for ID and non-EEG sleep metrics

GLM non-significant associations between PRS for ID and **(A)** subjective sleep quality (assessed by PSQI; N = 456; GLM: β=0.06, p=0.18; Spearman r=0.056 , p=0.24), **(B)** Daytime sleepiness (assessed by ESS; N = 456; GLM: β=-0.05, p=0.4; Spearman r=0.021 , p=0.46), **(C)** anxiety (assessed by BAI; N = 456; GLM: β=0.01, p=0.8; Spearman r=0.02 , p=0.65), **(D)** depression(assessed by BDI; N = 456; GLM: β=0.06, p=0.2; Spearman r =0.051 , p=0.3), and **(E)** probability of transition from rest to activity during night sleep (**kRA**)(Lim et al., 2011) (N = 359, subsample; GLM: β=-0.075, p=0.19; Spearman r=-0.038 , p=0.51) . PRS for ID was computed including SNPs with p-value threshold = 1. Spearman's correlations r are reported for completeness and do not replace GLM results. All metrics on the graphs including ID PRS (arbitrary units) are z-transformed before plotting. **PSQI**: Pittsburgh Sleep Quality Index (Buysse et al., 1989) ; **ESS**: Epworth sleepiness scale (Johns, 1991); **BAI**: Beck Anxiety Inventory (Beck et al., 1988) ; **BDI**: Beck Depression Inventory (A. T. Beck et al., 1988).

Table 4.S1. Sample characteristics (mean ± *SD or mean*)

N	456
Sex (men, women)	407, 49
Ethnicity	Caucasian
Age (years)	22.04 ± 2.68
Height (cm)	179.05 ± 8.08
BMI (kg m−2)	22.11 ± 2.32
Anxiety index	3.03 ± 3.46
Depression index	3.04 ± 3.45
Sleep quality	3.43 ± 1.76
Daytime sleepiness	5.68 ± 3.43
Baseline sleep duration (min)	449 ± 42
WASO (min)	13.3
SOL (min)	16.3
Arousals_REM (number)	21.4
SWE (µV^2)	2100591
Beta_NREM (µV^2)	17281
Theta_REM (µV^2)	81820

Anxiety and depression indices were estimated by Beck Anxiety Inventory (BAI) and Beck Depression Inventory (BDI) (A. T. Beck et al., 1988; Beck et al., 1988), Sleep quality and sleepiness were assessed with Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989)) and Epworth sleepiness scale (ESS) (Johns, 1991) respectively. **WASO**: wake after sleep onset; **SOL**: sleep onset latency; **Arousals_REM**: the number of arousals during REM sleep; **SWE**: slow wave energy (overnight cumulated delta - 0.5-4Hz – power) in NREM sleep. **Beta_NREM**: accumulated beta (16-25Hz) power in NREM sleep. **Theta_REM**: accumulated theta (4-8Hz) power in REM sleep.

Table 4.S2. Number of SNPs included in PRS computation as a function of p-value thresholding in reference GWAS summary statistic of (Jansen et al., 2019)

P Value threshold	Number of SNPs included in PRS computation
$5*10^{-8}$	198
$5*10^{-6}$	458
$5*10^{-4}$	2413
0.001	7142
0.01	27061
0.05	73962
0.1	116489
0.3	238845
0.5	327305
1	456750
All SNPs without LD prunning	7300849

Table 4.S3. Statistical outcomes of GLMs with the six baseline night sleep metrics of interest vs. PRS for ID from conservative ($p < 5x10^{-8}$) to inclusive ($p < 1$) p-value threshold and selecting all SNPs [Complementary to Figure 1].

Sleep metrics	P Value threshold for SNPs selection										
	$p=5*10^{-8}$	*$p=10^{-6}$*	*$p=10^{-4}$*	*p=.001*	*p=.01*	*p=.05*	*p=.1*	*p=.3*	*p=.5*	*p=1*	*All SNPs*
WASO N=453	ns										
SOL N=456	ns										
Arousals_REM N=452	ns			β=0.09 p=0.026 Sex β=0.54 p < 0.0001	β=0.09 p=0.021 Sex β=0.54 p < 0.0001	ns					
SWE_NREM N=453	ns		β=-0.09 p=0.045 Age β=-0.15 p = 0.0008 Sex β=-0.28 p < 0.0001 TST β=0.19 p < 0.0001	β=-0.10 p=0.018 Age β=-0.15 p = 0.0009 Sex β=-0.28 p < 0.0001 TST β=0.19 p < 0.0001	β=-0.12 p=0.009 Age β=-0.15 p = 0.0012 Sex β=-0.28 p < 0.0001 TST β=0.19 p < 0.0001	β=-0.12 p=0.006 Age β=-0.15 p = 0.0009 Sex β=-0.28 p < 0.0001 TST β=0.19 p < 0.0001	**β=-0.14** **p=0.002*** Age β=-0.15 p = 0.0009 Sex β=-0.28 p < 0.0001 TST β=0.19 p < 0.0001	β=-0.11 p=0.016 Age β=-0.15 p = 0.0008 Sex β=-0.29 p < 0.0001 TST β=0.19 p < 0.0001	**β=-0.15** **p=0.0007*** Age β=-0.15 p = 0.0008 Sex β=-0.29 p < 0.0001 TST β=0.19 p < 0.0001	**β=-0.16** **p=0.0003*** Age β=-0.15 p = 0.0008 Sex β=-0.29 p < 0.0001 TST β=0.19 p < 0.0001	β=-0.11 p=0.018 Age β=-0.15 p = 0.0012 Sex β=-0.28 p < 0.0001 TST β=0.19 p < 0.0001
Beta_NREM N=451	ns										
Theta_REM N=456	ns			β=-0.10 p=0.027 Age β=-0.13 p = 0.005 Sex β=-0.36 p < 0.0001 TST β=0.55 p < 0.0001	β=-0.10 p=0.017 Age β=-0.12 p = 0.006 Sex β=-0.36 p < 0.0001 TST β=0.55 p < 0.0001	β=-0.12 p=0.007 Age β=-0.12 p = 0.005 Sex β=-0.36 p < 0.0001 TST β=0.55 p < 0.0001	**β=-0.14** **p=0.0016*** Age β=-0.12 p = 0.005 Sex β=-0.36 p < 0.0001 TST β=0.55 p < 0.0001	**β=-0.13** **p=0.0026*** Age β=-0.12 p = 0.005 Sex β=-0.36 p < 0.0001 TST β=0.55 p < 0.0001	**β=-0.15** **p=0.0005*** Age β=-0.12 p = 0.004 Sex β=-0.36 p < 0.0001 TST β=0.55 p < 0.0001	**β=-0.15** **p=0.0003*** Age β=-0.12 p = 0.005 Sex β=-0.36 p < 0.0001 TST β=0.55 p < 0.0001	β=-0.10 p=0.017 Age β=-0.12 p = 0.006 Sex β=-0.36 p < 0.0001 TST β=0.55 p < 0.0001

GLMs included sex, age, BMI and TST. Only significant associations with p<.05 are reported. Partial effect sizes are provided for each significant association. ns: non-significant (p > 0.05).

***** association meeting study-wise correction for multiple comparisons (p < 0.0028).

SNPs: Single nucleotide polymorphisms; **SOL**: sleep onset latency; **WASO**: wake time after sleep onset; **Arousals_REM**: number of arousals in REM sleep; **SWE_NREM**: slow wave energy (overnight cumulated delta - 0.5-4Hz – power) in NREM sleep. **Beta_NREM**: accumulated beta (16-25Hz) power in NREM sleep. **Theta_REM**: accumulated theta (4-8Hz) power in REM sleep. The number of participants included in each GLM, following outliers removal and because of missing data, is reported below each dependent variable.

Table 4.S4. Statistical outcomes of GLMs between PRS for ID and with the SWE and cumulated theta power during REMS from conservative ($p < 5x10^{-8}$) to inclusive ($p < 1$) p-value threshold and selecting all SNPs

Sleep metrics	P Value threshold for SNPs selection										
	p=5*10^{-8}	***p =10^{--6}***	***p =10^{-4}***	***p=.001***	***p=.01***	***p=.05***	***p=.1***	***p=.3***	***p=.5***	***p=1***	***All SNPs***
SWE_NREM	ns		β=-0.09 p=0.05	β=-0.10 P=0.020	β=-0.12 P=0.010	β=-0.12 p=0.007	β=-0.13 P=0.003	β=-0.10 P=0.020	**β=-0.15 P=0.001***	**β=-0.16 P=0.0004***	β=-0.10 P=0.024
Theta_REM	ns			β=-0.09 P=0.025	β=-0.11 P=0.008	β=-0.12 P=0.005	**β=-0.13 P=0.002***	**β=-0.13 P=0.0025***	**β=-0.15 p=0.0005***	**β=-0.15 p=0.0003***	β=-0.10 P=0.018

GLMs included sex, age, BMI and TST (associations with these covariates are not reported for simplicity). Only significant association with p<.05 are reported. Partial effect sizes are provided for each significant association. ns: non-significant (p > 0.05).
SNPs: Single nucleotide polymorphisms; **SWE_NREM**: slow wave energy (overnight cumulated delta - 0.5-4Hz – power) in NREM sleep; **Theta_REM**: accumulated theta (4-8Hz) power in REM sleep.

Table 4.S5. Statistical outcomes of GLMs with the overnight cumulated power during REM and NREM sleep at baseline night vs. PRS for AD [complementary to Figure 2].

Cumulated power in baseline night	PRS for ID
Theta in NREM	**β=-0.14, p=0.0015***
Delta in REM	**β=-0.18, p=0.00004***
Sigma in NREM	**β=-0.11, p=0.009**
Sigma in REM	**β=-0.01, p=0.01**
Alpha in NREM	β=-0.05, p=0.25
Alpha in REM	β=-0.03, p=0.8
Beta in NREM	β=-0.003, p=0.9
Beta in REM	β=-0.01, p=0.8

Delta: 0.5-4Hz; Theta: 4-8Hz; Alpha: 8-12Hz; Sigma: 12-16Hz; Beta: 16-25Hz.
PRS for ID was computed including SNPs with p-value threshold = 1.

Table 4.S6. Statistical outcomes of GLMs with the cognitive metrics of interest vs. PRS for ID

Cognitive metrics	PRS for ID
Stroop task	β=-0.02, p=0.7
PASAT	β=-0.05, p=0.4
Memory of numbers MEM III - direct order	β=0.03, p=0.6
Memory of numbers MEM III - reverse order	β=0.01, p=0.8
List of words I MEM III - total score	β=-0.07, p=0.2
Family scenes II MEM III - total score	β=0.02, p=0.9

Stroop task is used to assess the ability to inhibit cognitive interference (Stroop, 1935).
Paced auditory serial addition test (PASAT) is a neuropsychological test used to assess capacity and rate of information processing and sustained and divided attention (Gronwall, 1977).
The remaining four tests are taken from Wechsler clinical scale of memory assessment MEM-III (Wechsler, 2001) and measure the ability to remember a number sequence in direct and reverse order (Memory of numbers MEM III), a list of words (List of words I MEM III) and scenes on the pictures (visual memory, Family scenes II MEM III).
PRS for ID was computed including SNPs with p-value threshold = 1.

Appendix 4

Supplemental figures and tables for the manuscript presented in Chapter 5.

***Table 5.S1**:* Statistical outcomes of GLMMs with the two sleep metrics of interest vs. the LC activity while controlling for the LC contrast

Sleep metric	***Group***	***LC activity***	***Age group***	***LC activity *group***	***Sex***	***BMI***	***TST***	***Contrast***
PSQI	all	**F(1,45) = 4.99** **P = 0.03***	F(1,45) = 1.31 P = 0.26	**F(1,45) = 6.78** **P = 0.012***	F(1,45) = 0.5 P = 0.48	F(1,45) = 0.77 P = 0.39		**F(1,45) = 5.34** **P = 0.025***
	young	t = -0.68 P = 0.49						
	old	**t = 2.53** **P = 0.015***						
Theta in REMS	all	F(1,44) = 2.89 P = 0.096	F(1,44) = 0.39 P = 0.5	**F(1,44) = 4.53** **P = 0.039***	F(1,44) = 0.01 P = 0.94	F(1,44) = 3.59 P = 0.65	F(1,44) = 0.73 P = 0.4	F(1,44) =0.05 P = 0.8
	young	t = 0.81 P = 0.42						
	old	**t = -2** **P = 0.05***						

The GLMs included sex, age, BMI and TST for the power metric. **PSQI**: Pittsburgh Sleep Quality Index (Buysse et al., 1989), **Theta in REMS**: accumulated theta (4-8Hz) power in REM sleep, **Contrast:** the LC contrast.