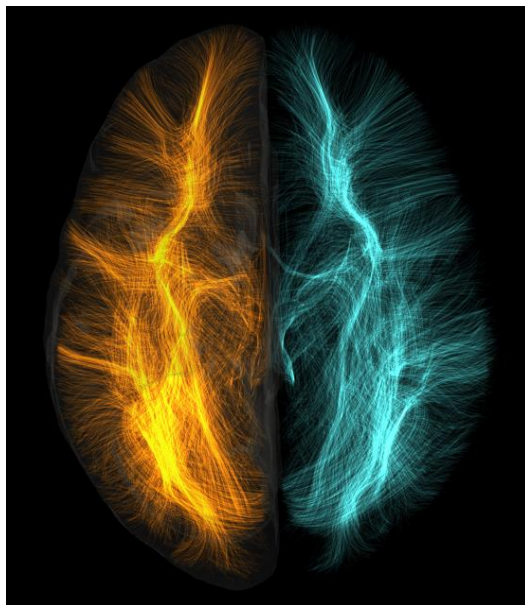


How temporal organization of neuronal function promotes successful cognitive aging



Maxime Van Egroo

Thesis presented for the academic degree of
Doctor in Psychological Sciences

December 2019

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To my father.

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List of abbreviations

AAL: automated anatomical labeling
A β : amyloid-beta
AD: Alzheimer's disease
ApoE: apolipoprotein E
APP: amyloid precursor protein
CEP: cortical excitability regulation profile
CSF: cerebrospinal fluid
DARTEL: diffeomorphic anatomical registration through exponentiated algebra
DMH: dorsomedial nucleus of the hypothalamus
DWI: diffusion-weighted imaging
EEG: electroencephalography
GABA: γ -aminobutyric acid
GLMM: generalized linear mixed model
GM: grey matter
hMRI: *in vivo* histology using magnetic resonance imaging
ISF: interstitial fluid
LC: locus coeruleus
MCI: mild cognitive impairment
MRI: magnetic resonance imaging
MT: magnetization transfer
NFT: neurofibrillary tangle
NREM: non-rapid eye movement
OSA: obstructive sleep apnea
PD: proton density
PET: positron emission tomography
R1: longitudinal relaxation rate
R2*: effective transverse relaxation rate
R²_p*: Semi-partial R²
REM: rapid eye movement
SCN: suprachiasmatic nucleus
SHY: synaptic homeostasis hypothesis

SUVR: standardized uptake value ratio

SWA: slow wave activity

SWE: slow wave energy

SWS: slow wave sleep

TMS: transcranial magnetic stimulation

Additional abbreviations used in figures and tables are defined in their corresponding legends.

Preface

Sleep is a central phenotype in all animal species, including humans. It is usually defined as a state of reduced activity and responsiveness to the environment, concomitant with changes in body physiology and brain electrophysiology. Unfortunately for sleep researchers, human adults spend approximately one third of their time in this state of inescapable inactivity, which leaves only two third of a lifetime to disentangle the complexity of sleep and its mechanisms.

For centuries, the fundamental aspect of sleep has urged thousands of scientists to try to decipher the ‘how’ and ‘why’ of sleep, be it regarding its physiological, psychological, sociological, or even philosophical features. Indeed, as stated by sleep researcher Dr. Rechtschaffen, *‘If sleep does not serve an absolute vital function, then it is the biggest mistake the evolutionary process ever made’* (Rechtschaffen, 1971, p. 88). Yet, despite a now well-established body of research on the processes underlying the generation and maintenance of a sleeping state (the ‘how’), we are still lacking critical understanding of the intrinsic reason of sleep (the ‘why’).

Assuming that sleep only serves a unique function would be far too simplistic, however. As pointed out by the plurality of models over the past decades regarding the role of sleep, it is clear that sleep embodies many functions spanning multiple branches in scientific research. Focusing on its intricate relationships with the brain, sleep is now described by neuroscientists as a necessary off-state allowing brain maintenance and favoring plasticity in order to optimize the subsequent period of wakefulness. According to this view, sleep therefore depends on, but also prepares, what is considered by definition as its polar opposite: wakefulness. Sleep and wakefulness are thus intertwined processes that feed and act on each other to form the intricate sleep-wake cycle which, as a whole, drives behavior and physiology. Yet, while thousands of researchers have emphasized the importance of studying the ‘sleep’ part, only a small amount of them have addressed its separate-but-interdependent ‘wake’ counterpart.

Importantly, regulation of both sleep and wakefulness shows marked changes across the human lifespan. From infancy to late-adulthood, sleep becomes less predominant and more fragmented while the quality of the wakefulness period is increasingly impacted. In older

individuals, an increasing amount of evidence shows that these age-related alterations of sleep-wake regulation are directly linked to cognition and pathophysiological processes in the brain. In our ever-aging/never-sleeping society, where neurodegenerative disorders such as Alzheimer's disease (AD) are expected to skyrocket over the next decades, re-considering the role of sleep-wake regulation and further characterizing its causes, correlates, and consequences, should constitute an imperative challenge for scientists as it will represent a crucial issue for public health.

In the present thesis, we will first introduce models of sleep-wake regulation and emphasize its role in cognitive aging and neurodegenerative diseases, with a particular attention for AD. Using state-of-the-art methods in the context of a multi-modal approach among healthy individuals in late middle-age (50-70 years), we will then show that preserved sleep-wake regulation is essential to successful aging, through three sets of published or submitted results. Mirroring the two thirds-one third distribution over the sleep-wake cycle, the results section of this thesis is divided into two chapters of results on wakefulness-related parameters while the last chapter will concentrate on sleep-derived features.

In the first chapter of results, we probed frontal cortical excitability dynamics during prolonged wakefulness using transcranial magnetic stimulation combined with high-density electroencephalography (TMS-EEG), and we assessed brain structural integrity using magnetic resonance imaging (MRI) as well as amyloid-beta and tau protein positron emission tomography (PET). Participants' performance over several cognitive domains was also extensively phenotyped. We show that cortical excitability dynamics are highly variable across individuals, with some older people displaying young-like regulation profile of cortical function. We further find that preserved wake-dependent regulation of cortical excitability is associated with cognitive fitness. Most importantly, these relationships are independent of age-related changes in brain structural integrity.

In the second set of results, we investigated the relationship between trait-like cortical excitability and PET tau protein burden in the brainstem monoaminergic grey matter including the locus coeruleus (LC), a small group of neurons involved in wakefulness regulation and the first site of tau pathology in AD. We report that frontal cortical hyper-excitability is associated with higher tau protein burden in the brainstem monoaminergic compartment, while it was not related to tau co-localizing in the hippocampus and entorhinal

cortex. Furthermore, using diffusion-weighted imaging (DWI) tractography, we show that frontal cortical excitability is specifically linked to the functional integrity of fronto-LC tracts.

In the last chapter of results, we measured the ability to produce sleep slow waves during a night of habitual sleep under EEG, and we used quantitative multi-parametric MRI to perform so-called *in vivo* histology (hMRI) of tissues in brain regions underlying sleep slow waves generation. We demonstrate that myelin content, as reflected by magnetization transfer measures, is higher in the frontal lobe of older individuals with a preserved ability to generate slow waves during sleep, suggesting a facilitated neuronal synchronization.

In a last section dedicated to the overall discussion, the integration and implications of these findings is further examined and put into perspective for future research, while emphasizing the strengths and limits of our experimental protocol.

We sincerely hope that this work will live up to the interest of the wonderful scientific field of chronobiology, and we wish the reader an informative and pleasant read.

Préface

Le sommeil est un phénotype central à tout le règne animal, y compris les humains. Il est généralement décrit comme un état d'activité réduite et de réactivité atténuée face à l'environnement, qui s'accompagne de modifications physiologiques corporelles et électrophysiologiques cérébrales. Malheureusement pour les chercheurs s'intéressant au sommeil, un adulte passe approximativement un tiers de sa vie dans cet état d'inexorable inactivité, ce qui ne laisse que deux tiers d'une existence humaine pour appréhender la complexité du sommeil et comprendre ses mécanismes.

Des siècles durant, l'aspect fondamental du sommeil a poussé une myriade de scientifiques à déchiffrer le 'comment' et le 'pourquoi' du sommeil, que ce soit en regard de ses composantes physiologiques, psychologiques, sociologiques, voire même philosophiques. En effet, comme énoncé par le chronobiologiste Dr. Rechtschaffen, *'Si le sommeil ne sert pas une fonction absolument vitale, alors c'est la plus grande erreur que l'évolution a commise.'* (Rechtschaffen, 1971, p. 88). Pourtant, alors que la littérature scientifique abonde quant aux processus sous-tendant la génération et le maintien d'un état de sommeil (le 'comment'), la raison intrinsèque de cet état nous échappe toujours (le 'pourquoi').

Supposer que le sommeil ne servirait qu'une seule et unique fonction serait bien trop simpliste, néanmoins. Tel que souligné par la pluralité de théories émises sur le rôle du sommeil durant les dernières décennies, il apparaît très clair que le sommeil incarne de nombreuses fonctions couvrant bien des domaines de la recherche scientifique. Plus particulièrement en ce qui concerne son étroite relation avec le cerveau, le sommeil est dorénavant décrit par les neuroscientifiques comme un état nécessaire qui permet une maintenance cérébrale et favorise la plasticité afin d'optimiser la période d'éveil subséquente. Selon cette théorie, le sommeil dépend de, mais surtout prépare, ce qui est par définition son opposé parfait: l'éveil. Le sommeil et l'éveil constituent dès lors un enchevêtrement de processus qui interagissent afin de former le cycle de veille-sommeil qui, dans son ensemble, régule le comportement et la physiologie. Cependant, alors que des milliers de chercheurs ont mis l'accent sur l'importance d'étudier la composante 'sommeil', seulement quelques centaines se sont focalisés sur l'aspect distinct mais interdépendant que représente 'l'éveil' dans cette dualité.

De manière importante, tant la régulation du sommeil que de l'éveil change profondément au cours de la vie. De l'enfance à la sénescence, le sommeil devient moins prédominant, plus fragmenté, tandis que la qualité de la période d'éveil s'amenuise. De plus en plus de données chez l'individu âgé montrent un lien direct entre ces modifications de la régulation veille-sommeil dépendantes de l'âge et la pathophysiologie du cerveau ainsi que la cognition. Dans nos sociétés où le sommeil perd peu à peu sa place, alors que la population vieillit et que la prévalence des maladies neurodégénératives s'accroît à toute allure, reconsidérer le rôle de la régulation veille-sommeil et caractériser davantage ses causes, ses corrélats, et ses conséquences devrait par conséquent s'avérer être un défi majeur pour les scientifiques et constituer un enjeu crucial pour la santé publique.

Dans le présent manuscrit, nous allons tout d'abord présenter des modèles de la régulation veille-sommeil et souligner son rôle dans le vieillissement cognitif et les maladies neurodégénératives telles que la maladie d'Alzheimer (MA). Dans le contexte d'une approche multimodale employant des techniques de pointe au sein d'individus âgés (50-70 ans), nous allons démontrer que la préservation de la régulation veille-sommeil est essentielle pour un vieillissement cognitif « réussi », tel qu'illustré par trois ensembles de résultats publiés ou soumis pour publication. A l'instar de la répartition deux tiers-un tiers dans le cycle de veille-sommeil, la section relative aux résultats est divisée en deux chapitres consacrés à des paramètres relatifs à l'éveil, tandis que le dernier chapitre se focalise sur une caractéristique dérivée du sommeil.

Dans le premier chapitre de résultats, nous avons mesuré la dynamique de l'excitabilité du cortex frontal durant l'éveil prolongé au moyen de la stimulation magnétique transcrânienne couplée à de l'électroencéphalographie haute densité (SMT-EEG), et nous avons également quantifié l'intégrité structurelle cérébrale grâce à l'imagerie par résonance magnétique (IRM) et la tomographie par émission de positrons (TEP) des protéines amyloïde-bêta et tau. Les performances des participants furent également recueillies au travers de différents domaines cognitifs. Nos résultats montrent que les dynamiques d'excitabilité corticale sont fortement variables d'un individu à l'autre, certaines personnes âgées présentant un profil de régulation de la fonction corticale « semblable aux jeunes ». Nous avons trouvé par ailleurs que de tels profils de régulation préservée de l'excitabilité corticale sont associés aux aptitudes cognitives. De manière critique, ces relations apparaissent indépendantes des modifications de l'intégrité structurelle du cerveau liées à l'âge.

Dans un deuxième ensemble de résultats, nous étudions la relation entre l'excitabilité corticale en tant que trait et la présence d'agrégats de protéine tau dans la matière grise monoaminergique du tronc cérébral, incluant le locus coeruleus (LC), un petit groupe de neurones impliqués dans la régulation de l'éveil et affecté précocement par la pathologie de la protéine tau au cours de la MA. Nous observons que l'hyperexcitabilité du cortex frontal est associée aux dépôts de protéine tau dans la partie monoaminergique du tronc cérébral, tandis qu'elle n'est pas liée au tau co-présent dans l'hippocampe et le cortex entorhinal. De plus, au moyen de techniques de tractographie basées sur l'imagerie par diffusion, nous montrons que l'excitabilité du cortex frontale est spécifiquement liée à l'intégrité fonctionnelle des faisceaux fronto-LC.

Dans le dernier chapitre des résultats, nous avons mesuré la capacité à produire des ondes lentes durant une nuit de sommeil typique sous EEG, et nous avons employé de l'IRM quantitative multiparamétrique afin d'approcher une histologie *in vivo* des tissus cérébraux sous-tendant la génération des ondes lentes. Nos résultats révèlent que le contenu en myéline, tel que mesuré par un indice relatif au transfert de magnétisation, est plus élevé dans le lobe frontal des individus âgés démontrant une capacité préservée à générer des ondes lentes durant le sommeil, suggérant une synchronisation neuronale facilitée.

Au travers de la dernière section dédiée à la discussion générale, nous examinons l'intégration et l'implication des résultats présentés, et nous abordons des perspectives de recherches futures tout en accentuant les forces et les faiblesses inhérentes à notre protocole expérimental.

Nous espérons sincèrement que le présent travail sera à la hauteur de l'intérêt du merveilleux domaine de recherche qu'est la chronobiologie, et nous souhaitons au lecteur une lecture instructive et agréable.

Theoretical introduction

The quest for the ‘How’ and ‘Why’ of sleep

Sleep is a defining feature of all animal species, with various degrees of predominance throughout the animal kingdom (Siegel, 2017). In humans, it is usually described as the combined influences of circadian and homeostatic regulatory mechanisms, which results in a reversible period of inactivity characterized by reduced response to environmental stimuli, altered metabolism, and dynamic changes in electroencephalographic (EEG) activity (Siegel, 2008). Throughout a typical sleep period, repeated cyclic patterns can be observed which involve two types of sleep defined by distinct EEG activity: non rapid-eye-movement (NREM) and REM sleep. More than a mere temporary state, sleep is a dynamical process involving temporally-ordered transient sub-states, imperfectly grasped under the notion of sleep stages, each possessing distinct EEG properties and controlled by the activity of specific sleep-wake regulating regions (R. E. Brown, Basheer, McKenna, Strecker, & McCarley, 2012; Scammell, Arrigoni, & Lipton, 2017).

NREM sleep comprises three stages reflecting a progressive slip into deeper sleep. Stage 1 features slow rolling-eye movements, low amplitude and mixed-frequency EEG activity involving mostly theta (4-8 Hz) and alpha (8-13 Hz) power bands, as well as the occasional presence of higher amplitude and short duration sharp waves (vertex). Sleep stage 2 corresponds to the onset of higher amplitude waves (K complex) and trains of transient faster frequency waves (sleep spindles, 12-16 Hz). A hallmark of stage 3 is the relative predominance of slow waves (0.5-4 Hz) with high peak-to-peak amplitude, especially noticeable in frontal regions. NREM sleep stage 3 is often referred to as ‘deep sleep’, because sensory gating blocks most of environmental stimuli to prevent awakenings during this stage (Hairston, Talbot, Eidelman, Gruber, & Harvey, 2010). By definition, REM sleep features rapid eye movements, but also muscular atonia. EEG activity during REM appears similar to what is observed during wakefulness, hence the terms ‘paradoxical sleep’ used when REM sleep was first described (Jouvet, 1965). Mixed-frequency activity of theta and alpha waves, although a bit slower than during wakefulness, is interleaved with 2-6 Hz sharply contoured triangular patterns (sawtooth waves). The experience of vivid, story-like dreams coincides with eye movements and takes place especially during REM sleep (Hobson, 2009).

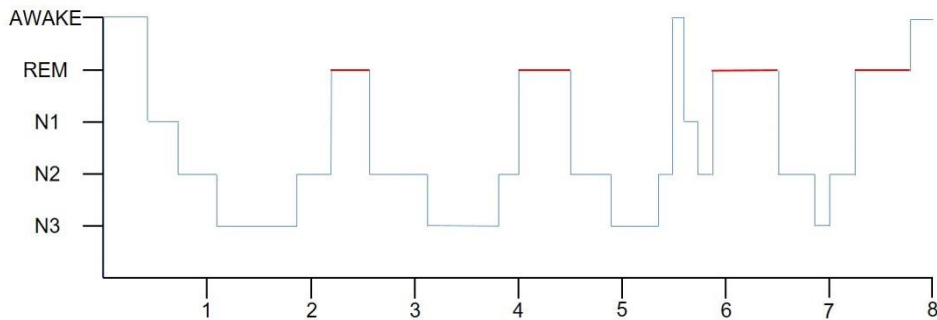


Figure 1. Prototypical profile of a night of sleep in a young adult aged 25 years. Multiple NREM-REM cycles occur over the course of the night, with NREM sleep duration gradually decreasing while REM sleep episodes become progressively longer. Courtesy of Daphne Chylinski.

A typical sleep cycle lasts around 90 minutes, with 4 to 6 cycles per night in young adults (McCarley, 2007) (**Figure 1**). On average, individuals should devote 8 hours to sleeping per 24h cycle (Hirshkowitz et al., 2015). For as long as it has existed, humankind has therefore spent approximately one third of its journey in a state of unavoidable torpor. During the remaining two-thirds of their time, sleep researchers joined efforts to disentangle sleep complexity regarding its (neuro-)physiological (Borbély, 1982; Nedergaard, 2013; Tononi & Cirelli, 2014), cognitive (Maquet, 2001; Walker, 2009), socio-cultural (Grandner, 2017; Rajaratnam & Arendt, 2001), or even its philosophical (Hill, 2004; Johnstone, 1973) dimensions. While a clear picture of the functions of sleep is still lacking (the ‘why’), decades of anatomical and physiological investigations have led to relatively well-described mechanisms underlying the regulation of wakefulness and sleep (the ‘how’). In the following section, we will focus on the ‘how’, using a key model of sleep-wake regulation and findings from neuroanatomy and genetics.

The ‘How’ of sleep-wake regulation

Modeling sleep-wake regulation

One landmark model of sleep and wakefulness regulation was proposed by Alexander Borbély in 1982 (**Figure 2**), and it has guided most of sleep research for the following decades up to now. The ‘two-process model of sleep regulation’ (Borbély, 1982) postulates that the timing of sleep and wakefulness is determined by the combined influences of the homeostatic process (Process S) and the circadian timing system (Process C).

The homeostatic process is a saturating exponential function of wakefulness duration which dissipates as an exponential function during sleep (Achermann & Borbély, 2003). It acts as an hourglass tracking the history of prior sleep and wakefulness periods to build or dissipate sleep need (Dijk & Archer, 2010). At the broad level of sleep architecture, partial and total sleep deprivation studies have robustly established that a longer duration of the wakefulness period leads to a compensatory increase in time spent in deeper sleep stage (*i.e.* NREM sleep stage 3) during the following sleep opportunity (Achermann, Dijk, Brunner, & Borbély, 1993; Berger & Oswald, 1962; Plante et al., 2016). EEG markers of the homeostatic ‘sleep debt’ consist in a progressive shift in power spectral density towards slower oscillations in the theta range during wakefulness (4-8 Hz), and in the delta range (0.5-4 Hz) during recovery sleep (Cajochen, Knoblauch, Kräuchi, Renz, & Wirz-Justice, 2001; Knoblauch, Kräuchi, Renz, Wirz-Justice, & Cajochen, 2002; Skorucak, Arbon, Dijk, & Achermann, 2018). At the molecular level, these markers are closely related to the accumulation of sleep-promoting substances, such as adenosine, in different sleep-wake regulating brain regions (Landolt, 2008; Retey et al., 2006). As experienced by everyone (especially PhD students in chronobiology), chronic sleep disruption or acute extended wakefulness also have strong implications at the behavioral level: individuals with heavy sleep need display decreased alertness, altered cognitive processing abilities, and significant mood worsening (Killgore, 2010; J. Lim & Dinges, 2010; Pilcher & Huffcutt, 1996).

The circadian system reflects the combined signals of all endogenous rhythms, physiological or behavioral, with a period of around 24h and synchronized by zeitgebers (time cues) such as the light-dark cycle (Buijs et al., 2016; Gaggioni, Maquet, Schmidt, Dijk, & Vandewalle, 2014). It consists in self-sustained pacemakers which contribute to the regulation

of the timing of sleep and wakefulness (Dijk & Archer, 2010). During the day, the circadian process increasingly promotes wakefulness to counteract the homeostatic build-up of sleep need. The circadian wake-promoting signals peak 2 to 3 h before habitual bedtime in what is called the ‘wake-maintenance zone’, a ~2h time window during which wakefulness is sustained although sleep pressure is considerably high (Strogatz, Kronauer, & Czeisler, 1987). In turn, during the biological night, circadian signals favor an extended and consolidated period of sleep in spite of the rapidly decreasing sleep debt. Well-established markers of the circadian process are melatonin (Dubocovich, 2007), cortisol (Bailey & Heitkemper, 2001), and core body temperature rhythms (Kolodyazhnyi et al., 2011). Similar to the homeostatic process, behavior is also partly modulated by the circadian system *per se*: forced desynchrony protocols, which consist in setting a sleep-wake periodicity outside the entrainment range of the circadian oscillator, have revealed a circadian regulation of alertness, mood, and several aspects of cognitive performance, with a general picture of better overall states during the biological day as opposed to the biological night (Dijk, Duffy, & Czeisler, 1992; Santhi et al., 2016; Schmidt, Collette, Cajochen, & Peigneux, 2007; Schmidt, Peigneux, & Cajochen, 2012).

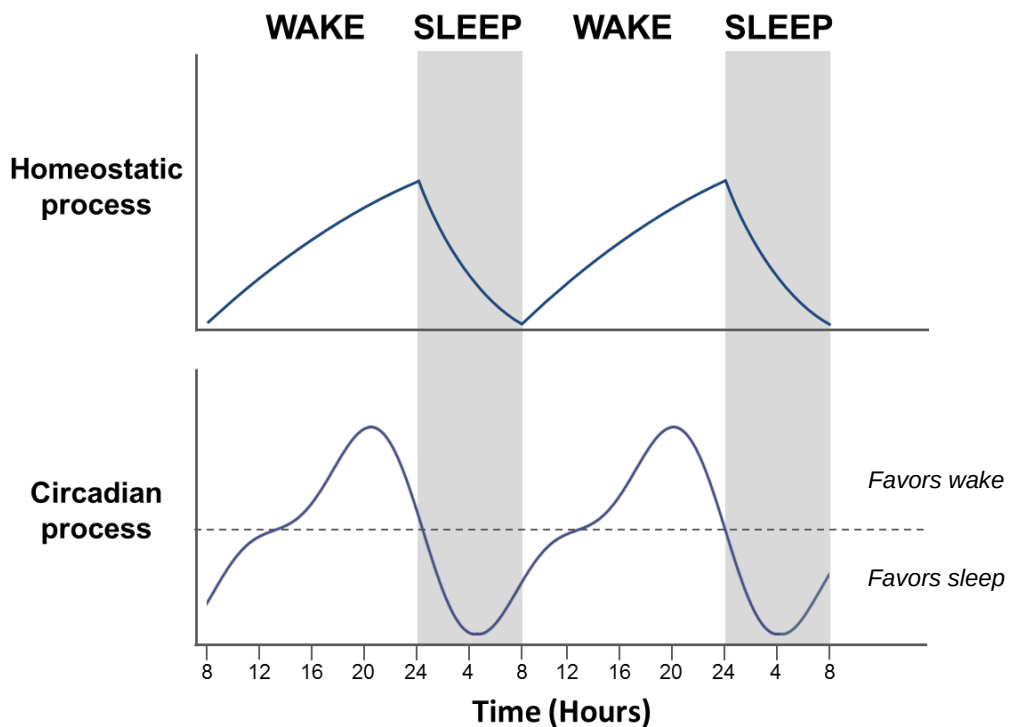


Figure 2. Theoretical illustration of the two components involved in sleep and wakefulness regulation according to the classical two-process model. The homeostatic process corresponds to an hourglass mechanism of physiological and behavioral modulations associated with increased wakefulness duration, which are dissipated during sleep. The circadian process reflects the combined outputs of all endogenous oscillatory near-24h circadian rhythms, independent from sleep or wakefulness duration. The interaction of these two factors regulates the timing of sleep and wakefulness and modulates cognition. Adapted from (Borbély, 1982).

The original two-process model stated that, more than their respective contributions alone, the interaction between process S and process C is especially crucial in determining two discrete events among the sleep-wake cycle, that is, the onset of sleep and wakefulness (Borbély, 1982). However, data suggested that a continuous interaction among these two processes over the 24-h cycle is more representative of experimental findings: circadian phase influences the amount of EEG NREM slow wave activity (SWA), a standard marker of sleep homeostasis (Dijk, 2009; Lazar, Lazar, & Dijk, 2015; Vyazovskiy, Achermann, & Tobler,

2007); sleep propensity throughout the sleep-wake cycle is modulated by the difference between process S and C (Dijk & Duffy, 1999; Fuller, Gooley, & Saper, 2006; Kräuchi & Wirz-Justice, 2001); sleep pressure influences the expression of circadian clock responses to time cues (Challet, Turek, Laute, & Van Reeth, 2001), and regulates the activity of the cerebral substrates underlying the circadian pacemaker (Schmidt et al., 2009). Besides, the classical model considered that the two-process regulation constituted a global phenomenon, acting on the brain in a topographical uniform manner. Yet, regional differences have been unveiled for both homeostatic and circadian components. Confined, use-dependent increase in NREM SWA has been observed over brain regions that were more solicited during prior wakefulness in humans (Huber, Ghilardi, Massimini, & Tononi, 2004), and occurrences of ‘local sleep’ were detected during prolonged wakefulness in rats (Vyazovskiy et al., 2011). Similarly, a topographical feature was highlighted in the relationship between circadian rhythmicity and characteristics of slow waves (Lazar et al., 2015). In addition, repeated functional MRI (fMRI) assessments during prolonged wakefulness in young adults found that task-driven brain responses displayed a locally modulated circadian rhythmicity (Muto et al., 2016).

Based on these, a reappraisal of the classical two-process model states that, at any time of day, physiology and behavior is defined not only by the independent influences of both sleep homeostasis and the circadian clock but also by their continuous interaction, and that these effects are expressed in a regional fashion (Borbély, Daan, Wirz-Justice, & Deboer, 2016).

Brain networks underlying sleep-wake regulation

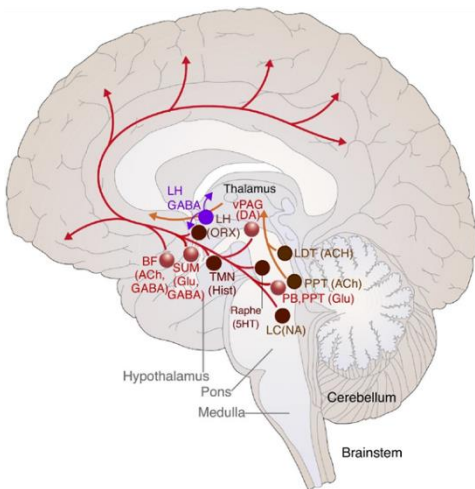
At the mechanistic level, sleep and wakefulness are regulated by the timely activity of a complex circuitry involving discrete but interdependent neuronal networks (Scammell et al., 2017). While EEG constitutes an important biomarker of sleep and wake states, neurochemical interactions between specific nuclei organized into wake- and sleep-promoting pathways are necessary to generate and sustain such states. Whereas initial investigations addressed the outcomes of localized brain lesions on the ability to sustain sleep or wakefulness, more recent studies used advanced methods of optogenetic or pharmacogenetic activation of specific nuclei to assess their role in sleep-wake regulation. Here, we will summarize the essential components of the circuitry underlying sleep-wake regulation that have been identified thus far (**Figure 3**).

The wake-promoting pathways involve the activity of monoaminergic cell groups mostly located in the brainstem, which display high firing rates during wakefulness, slower rates during NREM sleep, and are silent during REM sleep (Lu, Sherman, Devor, & Saper, 2006). Monoaminergic neurons provide the thalamus (dorsal pathway), and the basal forebrain, hypothalamus, and cortex (ventral pathway) with neurotransmitters such as norepinephrine (noradrenaline), serotonin, dopamine, or histamine that are crucial in driving arousal, processing sensory signals, and globally sustaining wakefulness (Eban-Rothschild, Appelbaum, & De Lecea, 2018). The major source of norepinephrine stems from the locus coeruleus (LC), a small group of neurons which display widespread projections to the entire brain (Jacobs, Müller-Ehrenberg, Priovoulos, & Roebroek, 2018; Priovoulos et al., 2018). LC neurons may be considered as a broadcast system which globally promotes arousal through divergent outputs to the brain. Serotonin in the basal forebrain originates from the dorsal and median raphe nuclei, and it promotes sleep-to-wake transitions by directly exciting other wake-promoting neurons (Ito et al., 2013). Dopaminergic neurons located in the ventral tegmental area bolster wakefulness, particularly when facing conditions of motivated behaviors (Eban-Rothschild, Rothschild, Giardino, Jones, & De Lecea, 2016). Histamine levels, solely dependent on the activity of the tuberomammillary nucleus, rise as soon as wakefulness begins and histaminergic neurons stop firing during sleep (Takahashi, Lin, & Sakai, 2006). In addition to the role played by monoaminergic neurons, wakefulness is also strongly promoted by glutamatergic and cholinergic neurons from the laterodorsal tegmental, parabrachial, pedunculopontine, and supramammillary nuclei which project to the basal forebrain (Kroeger et al., 2017; Pedersen et al., 2017). Critically, wakefulness may be primarily regulated by a hierarchically-organized fast neurotransmitter system located in the basal forebrain, which includes glutamatergic, cholinergic, and parvalbumin-containing γ -aminobutyric acid (GABA)-ergic neurons that innervate the cerebral cortex and are able to induce wakefulness (Saper & Fuller, 2017; Xu et al., 2015). Finally, all these wake-promoting regions are further excited by orexinergic neurons located in the lateral hypothalamus (Sasaki et al., 2011). A disruption of the orexin system, such as observed in narcolepsy, strongly impedes the maintenance of wakefulness and triggers intermittent transitions from wake to sleep states (Mahoney, Cogswell, Koralnik, & Scammell, 2019).

The sleep-promoting circuit relies on different brain substrates for NREM and REM sleep. Regulation of NREM sleep involves GABAergic neurons from the median and ventrolateral preoptic area. Through direct axonal projections, these sleep-active neurons

exhibit a mutual inhibition pattern with most actors of the wake-promoting system, resulting in a so-called ‘flip-flop’ mechanism which enables rapid transitions from wakefulness to sleep and vice-versa (Saper & Fuller, 2017; Saper, Fuller, Pedersen, Lu, & Scammell, 2010). Interestingly, increasing homeostatic sleep pressure is associated with faster firing rates of neurons in the median and ventrolateral preoptic area (Alam, Kumar, McGinty, Alam, & Szymusiak, 2014). GABAergic neurons from the parafacial zone, located in the lower brainstem, specifically act on the parabrachial nucleus to promote sleep and are required for the generation of slow wave sleep (Anaclet et al., 2014). In addition, some GABAergic neurons from the basal forebrain directly inhibit nearby wake-promoting neurons to promote NREM sleep (Xu et al., 2015). On the other hand, REM sleep is generated by glutamatergic neurons situated inferior to the LC, termed sublateralodorsal or subcoeruleus nucleus (Fraigne, Torontali, Snow, & Peever, 2015), as well as cholinergic neurons of the laterodorsal tegmental and pedunculopontine nuclei (Scammell et al., 2017). Subcoeruleus glutamatergic neurons control motor atonia through the activation of inhibitory interneurons in the medulla and spinal cord. The neural basis of the mixed frequency activity observed during REM sleep may originate from cholinergic REM active cell groups also involved in wakefulness (parabrachial, pedunculopontine and laterodorsal tegmental nuclei). REM sleep is suppressed during wakefulness and NREM sleep thanks to the activity of inhibitory neurons situated in the ventrolateral periaqueductal grey. In turn, these neurons are inhibited by subcoeruleus glutamatergic cells during REM sleep.

Wake-promoting pathways



Sleep-promoting pathways

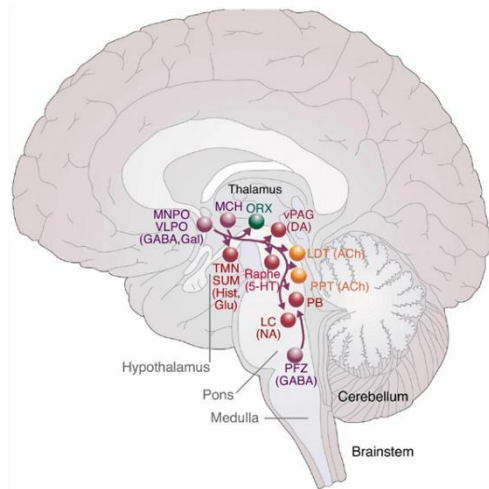


Figure 3. Neurochemical circuits underlying wake- and sleep-promoting pathways. Wakefulness is generated and maintained by the activity of monoaminergic, glutamatergic, and cholinergic neurons located in the upper brainstem, basal forebrain, and hypothalamus. NREM sleep is mainly sustained by GABAergic neurons from the median and ventrolateral preoptic area. REM sleep and its characterizing features arise from the activity of glutamatergic neurons located in the subcoeruleus region and cholinergic cells from the laterodorsal tegmental and pedunculopontine nuclei. ACh: acetylcholine; BF: basal forebrain; DA: dopamine; GABA: γ -aminobutyric acid; Hist: histamine; Glu: glutamate; LC: locus coeruleus; LDT: laterodorsal tegmental nucleus; LH: lateral hypothalamus; MCH: melanin-concentrating hormone; MNPO: median preoptic area; NA: noradrenaline; ORX: orexin; PB: parabrachial nucleus; PFZ: parafacial zone; PPT: pedunculopontine tegmental nucleus; SUM: supramammillary nucleus; TMN: tuberomammillary nucleus; VLPO: ventrolateral preoptic area; vPAG: ventral periaqueductal grey matter; 5HT: serotonin. Adapted from (Saper & Fuller, 2017).

Finally, while the aforementioned pathways are regulating short-term transitions from wakefulness to NREM/REM sleep states, the circadian regulation of the sleep-wake cycle over the 24h day-night period relies on the suprachiasmatic nucleus (SCN). The SCN comprises a pair of nuclei located in the anterior hypothalamus, above the optic chiasm, which are directly linked to the retinohypothalamic tract (Gaggioni et al., 2014). As mentioned earlier, the SCN acts as a central pacemaker; a ‘master clock’ synchronizing endogenous molecular, cellular, and behavioral rhythms with environmental stimuli, such as the solar cycle (Moore, 2007). It is composed of a ‘core’ including vasoactive intestinal peptide co-

expressing neurons, and a ‘shell’ containing arginine vasopressin expressing neurons (Scammell et al., 2017). The core and the shell interact to form a self-sustained circadian oscillator, which remains functional in the absence of external stimuli (Mieda et al., 2015). Regulation of sleep and wakefulness by the SCN is mediated through its significant efferent connections to the subparaventricular zone, which in turn displays heavy projections to the dorsomedial nucleus of the hypothalamus (DMH). Eventually, wakefulness is promoted by the glutamatergic neurons of the DMH by activating the wake-promoting nuclei described above. By contrast, GABAergic neurons in the DMH innervate sleep-promoting cell groups such as the medial and ventrolateral preoptic area (Vujovic, Gooley, Jhou, & Saper, 2015).

Genetics of sleep-wake regulation

Extensive research on the genetics of sleep-wake regulation have led to a relatively comprehensive description of a core set of genes underlying the contribution of the circadian process to the temporal organization of the sleep-wake cycle (Dijk & Duffy, 1999; Duncan et al., 2012; Franken & Dijk, 2009; Lane et al., 2016; Logan & McClung, 2018). However, unravelling the identity of genes that regulate sleep homeostasis and its characteristics remains a continuous research effort (Allada, Cirelli, & Sehgal, 2017; Donlea, Alam, & Szymusiak, 2017; Eban-Rothschild et al., 2018; Funato et al., 2016; Liu et al., 2014; Mang & Franken, 2015).

The SCN-driven circadian regulation of sleep and wakefulness described above relies on a complex molecular mechanism present in almost every cells of the human body, and which involves a transcriptional-translational feedback loop (Blum, Bell, & Wu, 2018). In brief, this ‘circadian clock’ involves first the expression and dimerization of positive elements, CLOCK and BMAL1 (or NPAS2), which drives the transcription of *PERIOD* and *CRYPTOCHROME* genes. In turn, a negative feedback is provided by PERIOD and CRYPTOCHROME proteins dimerization which inhibits their own transcription by acting on CLOCK, NPAS2, and BMAL1 activity (Ko & Takahashi, 2008). Finally, post-translational protein degradation enables the beginning of a new cycle by removing such inhibition. Circadian rhythmicity in gene expression is therefore an emerging property of this network of transcriptional regulators, termed ‘clock genes’, and the role of the SCN is to orchestrate all peripheral circadian oscillators (Franken & Dijk, 2009).

Interestingly, variants among these clock genes have been linked to non-pathological inter-individual differences in certain sleep-wake characteristics, but also with clinical disorders of the sleep-wake cycle (Hamet & Tremblay, 2006; Sehgal & Mignot, 2011). These data inform about candidate mechanisms of the genetic regulation of sleep and its characteristics: for example, regulation of daily sleep length is influenced by a mutation in *DEC2* gene coding for a transcription repressor associated with natural shorter sleep duration, as demonstrated by animal models carrying the human mutation (He et al., 2009). Particularly, the human variable number tandem repeat polymorphism in the *PERIOD3* gene has been associated with diurnal/nocturnal preference, sleep timing, REM and NREM sleep regulation, as well as wakefulness-dependent EEG modulations (Dijk & Archer, 2010). Narcolepsy, a condition involving excessive daytime sleepiness and brief emotion-driven episodes of cataplexy, is related to a susceptible genetic background of variants in autoimmune-related genes which end up damaging orexinergic neurons in the hypothalamus (Hor et al., 2010; Sehgal & Mignot, 2011). On the opposite, predisposing genetic factors for clinical insomnia are not clear but may include mutations in the gene coding for a GABA subunit, pointing to potential deficits in inhibition in these patients (Palagini, Biber, & Riemann, 2014). Familial delayed sleep phase disorder, a condition characterized by a marked delay in the timing of sleep and wakefulness onset, have been linked to mutation of *PERIOD3* and *CRY1* genes. By contrast, familial advanced sleep phase syndrome, featuring persistent early sleep and wakefulness timing, was associated with a mutation in the *PERIOD2* gene (Reid et al., 2001; Toh et al., 2001).

Sleep-wake regulation in aging

In the previous sections, we depicted sleep-wake regulation as a universal process based on the interactions between neuroanatomical, molecular, and genetic components. Yet, there are marked changes in sleep and wakefulness across the human lifespan that are particularly evident when looking at the distribution and the duration of sleep and wake periods in the 24h cycle. Newborns sleep approximately 16 to 18 hours per day in a polyphasic manner, that is, through multiple and irregularly distributed periods, mostly regardless of daytime or nighttime (K. F. Davis, Parker, & Montgomery, 2004). In early infancy, REM sleep constitutes up to 50% of the sleep periods (Skeldon, Derks, & Dijk, 2016). During infancy and childhood, total sleep duration gradually decreases and periods of sleep and wakefulness become more distinct, while daytime napping is progressively abandoned (Carter

& Wrede, 2017). Concomitantly, REM sleep is reduced to the adult level of around 20-25% (Bathory & Tomopoulos, 2017). Adolescence is associated with a typical phase delay in the circadian timing system and a greater tolerance to accumulating sleep debt, resulting in a tendency towards later bedtimes (Tarokh, Saletin, & Carskadon, 2016). Sleep in early adolescence is particularly rich in NREM SWA, but a strong decline is observed between ages 11 and 17 years (Feinberg & Campbell, 2010). From young adulthood to older age, several macrostructural 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 (Scullin, 2017; Scullin & Bliwise, 2015). Older individuals have earlier bedtimes and wake-up times (*i.e.* phase advance), display a shorter and more fragile sleep period, spend more time awake during the night as well as more time asleep during the day (*i.e.* fragmentation of sleep and intrusion of daytime napping), and exhibit an increasing predominance of lighter sleep stages in their sleep architecture (**Figure 4**) (Mander, Winer, & Walker, 2017).

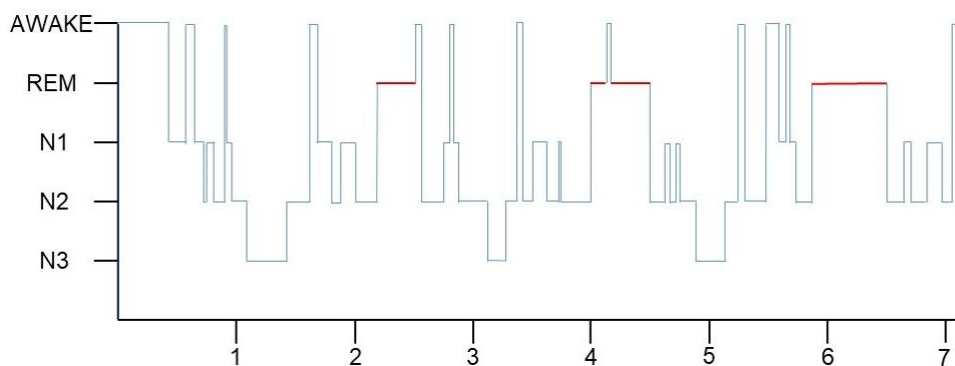


Figure 4. Typical profile of a night of sleep in an older individual aged 60 years. With increasing age, sleep becomes lighter and more fragmented, accompanied by frequent nocturnal awakenings. Compared with their younger counterparts, older adults generate less slow wave sleep and spend less time in REM sleep. Courtesy of Daphne Chylinski.

In light of the two-process model of sleep-wake regulation, it is now well-established that the late-life age-related changes in the sleep-wake cycle are the result of combined alterations in both the homeostatic process and the circadian system (**Figure 5**). The homeostatic sleep pressure dissipation slope becomes slower and shallower, suggesting a reduced build-up of sleep need in aging (Cajochen, Münch, Knoblauch, Blatter, & Wirz-Justice, 2006; Münch et al., 2004). Starting at around the 5th decade of life, the homeostatic marker of NREM SWA is gradually decreasing with maximal effects of age being observed over the frontal cortex (Carrier et al., 2011; Mander et al., 2013). Of note, the age-related reduction in NREM SWA is in part gender-specific: men aged over 70 years generate three times less slow wave sleep compared to their female counterparts, and the latter do not display significant differences in the amount of slow wave sleep relative to women aged 55 years or less (Mander et al., 2017; Ohayon, Carskadon, Guilleminault, & Vitiello, 2004). The circadian system is not spared by the aging process either: while their period remain somewhat unaffected, most circadian rhythms show a reduced amplitude in older people as well as an advance in phase of approximately 2 hours (Cajochen et al., 2006; Dijk, Duffy, Kiel, Shanahan, & Czeisler, 1999; Münch et al., 2005). In addition, these circadian oscillations become more difficultly entrained by environmental time cues (Farajnia, Deboer, Rohling, Meijer, & Michel, 2014).

Importantly, the underlying sleep- and wake-promoting pathways described above are also subject to changes when growing older. Desynchronization and limited cell loss within SCN neurons (Farajnia et al., 2014, 2012; Palomba et al., 2008; J. L. Wang et al., 2015), alteration of orexinergic and melanin-concentrating hormone neurons of the hypothalamus and their projections (Brownell & Conti, 2010; J. Mattis & Sehgal, 2016), reduction in axonal density among LC neurons and cholinergic neurons of the nucleus basalis of Meynert (Manaye, McIntire, Mann, & German, 1995; Mather & Harley, 2016; Nyakas, Granic, Halmy, Banerjee, & Luiten, 2011); all these structural degradation of sleep-wake regulation circuits may contribute to the age-related modifications in sleep and wakefulness phenotypes.

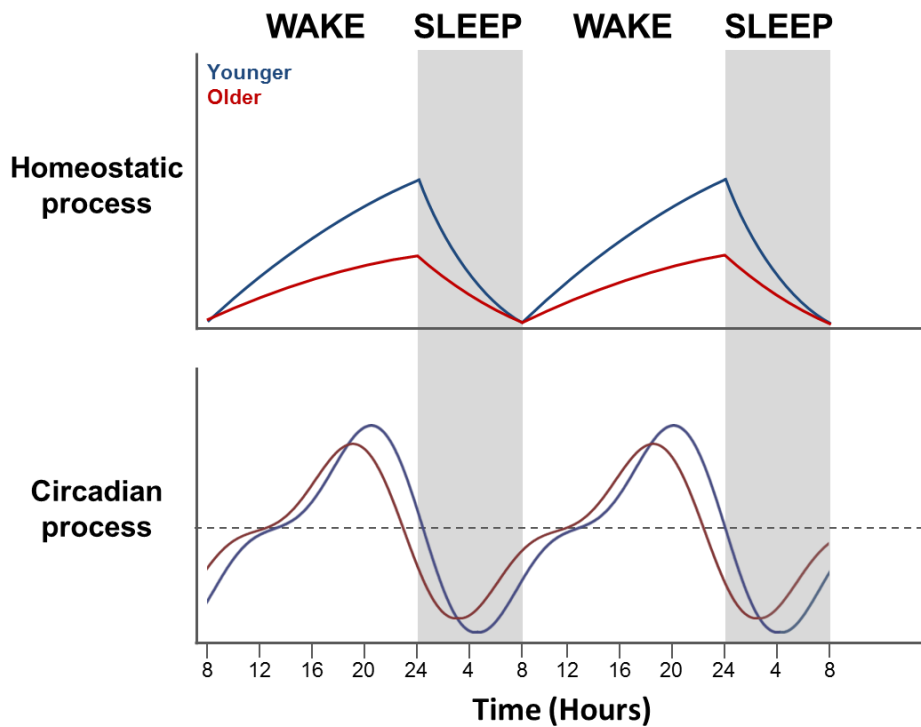


Figure 5. Theoretical representation of age-related changes in sleep-wake regulation in the context of the two-process model. With increasing age (red curves), the wakefulness-dependent build-up of sleep need is reduced and its sleep-dependent dissipation is less steep. The circadian timing system shows a reduced amplitude compared to younger individuals, as well as an advance in phase. The combined effects of these age-related changes is reflected in a fragmentation of the sleep-wake cycle, with earlier timing of sleep and wakefulness onset compared to young individuals. Modified from (Borbély, 1982).

The ‘Why’ of sleep-wake regulation

In spite of sleep being a shared experience among all humans, research is still trying to figure out the ‘why’ of sleep, that is, the intrinsic reason(s) underlying the evolutionary need for such a phenotype (Allada & Siegel, 2008; Frank, 2006; Krueger, Frank, Wisor, & Roy, 2016; Siegel, 2005). To date, several functions have been attributed to sleep: memory consolidation (Maquet, 2001), brain metabolites clearance (Mendelsohn & Larrick, 2013), synaptic homeostasis (Tononi & Cirelli, 2014), energy conservation (Scharf, Naidoo, Zimmerman, & Pack, 2008), metabolism regulation (Leger, Bayon, & de Sanctis, 2015).

In this section, we will focus on two concepts that are of particular interest in the context of our work: sleep-wake regulation as a critical factor in neurodegenerative diseases, and sleep as a necessary off-state to optimize wakefulness.

Sleep-wake regulation and neurodegenerative diseases

Disruption of sleep and wakefulness constitute a major symptom in a large range of neurodegenerative disorders, including Alzheimer’s disease (AD) (Musiek, Xiong, & Holtzman, 2015), Parkinson’s disease (Dauvilliers, 2007), Huntington’s disease (Morton et al., 2005), and tauopathies such as progressive supranuclear palsy (Walsh et al., 2017). Especially, the bidirectional relationship between AD and sleep-wake regulation has been at the core of a considerable amount of research efforts over the recent years (Gagnon, Lafrenière, Rauchs, Petit, & Carrier, 2019; Shi et al., 2018). As described in more details below, the rise of sleep-wake disturbances to the status of potential principal actor in AD pathogenesis was partially driven by the recent discovery of the glymphatic system (Nedergaard, 2013), a brain metabolite clearance process which was reported to be promoted by sleep (**Figure 6**) (Fultz et al., 2019; Jessen, Munk, Lundgaard, & Nedergaard, 2015; Rasmussen, Mestre, & Nedergaard, 2018). Hereafter, we review evidence in the literature for a significant interplay between the hallmarks of AD pathogenesis and disturbances in sleep and wakefulness.

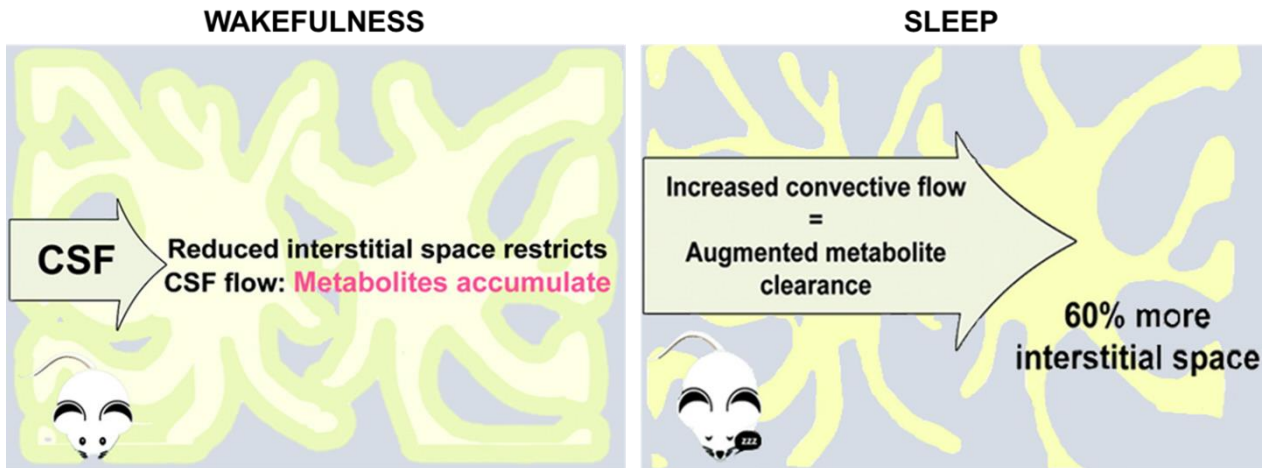


Figure 6. Illustration of the sleep-driven glymphatic clearance of brain metabolites. Wakefulness is associated with restrained interstitial space, impeding the glymphatic bulk flow and thus reducing clearance of potentially toxic neuronal waste. During sleep, the interstitial space is up to 60% larger, facilitating glymphatic flow and metabolite clearance. Modified from (Mendelsohn & Larrick, 2013).

Sleep-wake regulation and the hallmarks of the pathogenesis of Alzheimer's disease

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Abstract

While efficient treatments for Alzheimer's disease (AD) remain elusive, a growing body of research has highlighted sleep-wake regulation as a potential modifiable factor to delay disease progression. Evidence accumulated in recent years is pointing toward a tight link between sleep-wake disruption and the three main hallmarks of the pathogenesis of AD, *i.e.* abnormal amyloid-beta ($A\beta$) and tau proteins accumulation, and neurodegeneration. However, all three hallmarks are rarely considered together in the same study. In this review, we gather and discuss findings in favor of an association between sleep-wake disruption and each AD hallmarks in animal models and in humans, with a focus on the preclinical stages of the disease. We emphasize that these relationships are likely bidirectional for each of these hallmarks. Altogether, current findings provide strong support for considering sleep-wake disruption as a true risk factor in the early unfolding of AD, but more research integrating recent technical advances is needed, particularly with respect to tau protein and neurodegeneration. Interventional longitudinal studies among cognitively healthy older individuals should assess the practical use of improving sleep-wake regulation to slow down the progression of AD pathogenesis.

Introduction

The current search for an Alzheimer's disease (AD) treatment, although encouraging, is still failing to provide efficient therapies to significantly delay disease progression, and the existing ones only grant marginal symptomatic relief (Scheltens et al., 2016). Indeed, several recent clinical trials were unsuccessful in developing new drug therapies against AD (Cummings et al., 2016; Mangialasche, Solomon, Winblad, Mecocci, & Kivipelto, 2010). With an estimated health-care cost of 818 billion US dollars per year worldwide, AD, the most prevalent form of dementia, represents an important socio-economic and scientific challenge in our ever-aging society (Prince et al., 2015). Some consider that delaying the onset of AD by just 5 years would decrease AD costs by 40% (Zissimopoulos, Crimmins, & St.clair, 2015). Early detection and prevention are promising means to reach this objective (Mather & Harley, 2016). However, only 30% of AD cases are considered to be associated with recognized environmental risk factors for AD (diabetes, low education, smoking, lack of physical activity, hypertension, and depression) and acting on them would only moderately attenuate the foreseen increase in AD prevalence (Norton, Matthews, Barnes, Yaffe, & Brayne, 2014). Hence, identifying novel modifiable protective and risk factors for AD is crucially needed. These novel factors should facilitate the early detection of an increased risk for AD, even in asymptomatic individuals, and allow for more efficient prevention (Mather & Harley, 2016). In the present review paper, we argue that the regulation of the sleep-wake cycle and its disruption constitute such novel factors. The last decade has indeed seen a growing body of evidence supporting the link between AD-related processes and the regulation of sleep and wakefulness (Benedict et al., 2015; Hahn, Wang, Andel, & Fratiglioni, 2014; Landry & Liu-Ambrose, 2014; A. S. Lim, Kowgier, Yu, Buchman, & Bennett, 2013; Musiek & Holtzman, 2016). Accordingly, a recent meta-analysis of 27 observational studies revealed that approximately 15% of AD in the population may be attributed to treatable sleep problems, and that individuals with sleep-related issues exhibit a 1.55 times higher risk of developing the disease (Bubu et al., 2017).

The main pathophysiological hallmarks of AD are the abnormal accumulation in the brain of amyloid-beta ($A\beta$) protein in plaques and of tau protein in neurofibrillary tangles (NFTs), together with neurodegeneration, i.e. synaptic and neuronal loss (Jack et al., 2010). These hallmarks contribute to predicting the conversion of mild cognitive impairment (MCI), a prodromal phase of AD, to fully declared AD (Jack et al., 2013; Roe et al., 2013). Importantly, it is now well established that $A\beta$ and tau protein accumulations as well as brain

atrophy start decades before MCI or AD symptoms onset. A period corresponding to AD ‘preclinical’ stages has therefore been proposed and encompasses the 10 to 20 years preceding observable cognitive impairments (Jack et al., 2013; Sperling et al., 2011). It is important to stress that AD preclinical stages reflect an increased risk for the disease rather than being a deterministic feature: whether and when one will develop symptomatic AD cannot be established based solely on the presence of AD pathophysiological hallmarks. Nevertheless, together with Apolipoprotein E (*APOE*) polymorphism, the strongest genetic risk factor for sporadic AD (Kim, Basak, & Holtzman, 2009; Michaelson, 2014; Poirier et al., 1993), these pathophysiological changes offer the current best bases for applying prevention strategies to individuals at risk of developing sporadic AD.

Here, we provide a narrative review of the evidence for the relationship between the disruption of the sleep-wake cycle and the hallmarks of AD (i.e. A β burden, tau burden, and neurodegeneration) during its preclinical stages, both in humans and animal models. The papers linking sleep-wake regulation and AD pathophysiology that were considered are summarized in an overview table for each hallmark, i.e. at the end of the A β section (**Table 1**), of the tau section (**Table 2**), and of the neurodegeneration section (**Table 3**). We will only discuss the late-onset sporadic form of AD as it represents the vast majority of AD cases compared with the early-onset familial form which accounts for less than 5% of AD cases (Tanzi, 2012; X.-C. Zhu et al., 2015).

Sleep-wake regulation and amyloid-beta

A β refers to peptides of 36-43 amino acids that normally exist in the brain in a soluble form but become toxic when aggregated into diffusible oligomers (Masters & Selkoe, 2012) and eventually form extracellular insoluble amyloid plaques (De Strooper, Vassar, & Golde, 2010). In non-pathological conditions, the physiological role of A β is uncertain but is suspected to participate to normal synaptic function (Masters & Selkoe, 2012). A β 40 is the major A β species (~80-90%) produced by β - and γ -secretase in the sequential enzymatic cleavage of the larger transmembrane amyloid precursor protein (APP). A β 42, one of the minor species (~5-10%), plays a crucial role in the aggregation of amyloid into plaques because of its hydrophobic and fibrillogenic properties (Kim et al., 2007; M. P. Lambert et al., 1998; Murphy & LeVine III, 2010). One can monitor AD pathophysiology progression with positron emission tomography (PET) using A β ligands, but A β 42 assay in the cerebrospinal fluid (CSF) is also a sensitive and common biomarker for AD. In fact, lower

CSF A β 42 concentration is thought to reflect and even precede amyloid plaque formation in the brain (Palmqvist, Mattsson, & Hansson, 2016; Sprecher et al., 2017).

The AD-related pathophysiological process of A β follows a stereotyped progression pattern, with A β deposits first found in the neocortex. As the disease progresses, allocortical brain regions (e.g. entorhinal cortex, hippocampus) are involved as well as nuclei from the basal forebrain. Ultimately, A β burden encompasses brainstem nuclei and the cerebellum (Thal, Rüb, Orantes, & Braak, 2002). The prevailing theory of AD pathogenesis hypothesizes that the accumulation of A β into insoluble plaques is the key initiator of a series of pathogenic processes that eventually lead to AD (Hardy & Higgins, 1992; Hardy & Selkoe, 2002). At first, changes in A β levels caused by an increase in total A β production and/or a reduced clearance result in A β deposits that impede synaptic function and diminish long-term potentiation of neuronal circuits. Inflammatory responses and plaque formation further amplify synaptic and neuronal damage, precipitating widespread neuronal dysfunction, cell death, and ultimately dementia with plaque and tangle pathology (Haass & Selkoe, 2007). Although the broad outlines of the so-called ‘amyloid cascade hypothesis’ have been supported by the work of many researchers, whether A β accumulation is the prime event in the development of AD neuropathological processes is still not clearly established (Drachman, 2014; Karran, Mercken, & Strooper, 2011; Marchesi, 2011; Musiek & Holtzman, 2015; Simic et al., 2009). Furthermore, soluble forms of A β , i.e. the precursors that subsequently lead to A β plaques, might be more toxic than the aggregated insoluble forms: oligomeric neurotoxic species of A β bind to different components of neuronal and non-neuronal plasma membranes, and induce complex patterns of synaptic dysfunction and synapse loss (Masters & Selkoe, 2012).

The striking role of the sleep-wake cycle in the unfolding of A β pathology finds its roots in two landmark findings: the discovery that soluble A β dynamics are strongly associated with sleep and wakefulness (Huang et al., 2012; Kang et al., 2009), and the existence of a sleep-related metabolite clearance system in the brain (Mendelsohn & Larrick, 2013; Xie et al., 2013). Using *in vivo* microdialysis, Kang and colleagues (Kang et al., 2009) investigated hippocampal A β levels during wakefulness and sleep in wild-type mice and Tg2576 mice, a well-characterized mouse model of AD known to overexpress a mutant form of human APP (Hsiao et al., 1996; Westerman et al., 2002). First, they found that interstitial fluid (ISF) A β levels of Tg2576 mice exhibit a diurnal variation, with a significant decrease of approximately 25% during the light period (i.e. when mice sleep most) relative to the dark

period. Crucially, they observed that the same pattern of variation is also present in the ISF of wild-type mice and in the CSF of young healthy humans, indicating that A β fluctuation over a 24h-period is inherent to normal cellular physiology. Furthermore, sleep-deprived mice showed significantly increased A β levels that were promptly reduced after sleep recovery. In order to unravel the underlying molecular mechanisms, the potential role of orexin, a peptide promoting arousal and wakefulness (Chemelli et al., 1999; Mahler, Moorman, Smith, James, & Aston-Jones, 2014; Roh et al., 2014), was put forward. Interestingly, orexin infusion increased ISF A β levels during the light period while an orexin receptor antagonist abolished the previously observed diurnal variation. At a broader level, chronic sleep restriction in mice (20 hours daily for 21 days) was associated with greater A β plaque burden among several brain regions, whereas enhancing sleep via chronic orexin antagonist treatment (once daily for 8 weeks) was related to reduced A β plaque deposition.

The characterization of the sleep-wake modulation of A β dynamics was further expanded to include a recently described process of brain cellular waste regulation, called the 'glymphatic system' (Nedergaard, 2013; Xie et al., 2013). This system consists in convective fluxes from the para-arterial CSF through ISF and toward the para-venous space that allow neuronal products to be transported to the systemic circulation for clearance (Nakada & Kwee, 2018; Nedergaard, 2013). The glymphatic process can be altered by genetic variations affecting aquaporin 4 water channels (Rainey-Smith et al., 2018) and has been linked to AD and other dementias (Rasmussen et al., 2018). Using two-photon imaging of the brain of living adult mice, Xie et al. (Xie et al., 2013) found that the glymphatic system is strongly regulated by the sleep-wake cycle. They observed that the CSF influx was highly increased in the brain of sleeping and anesthetized mice compared to awake littermates. In turn, the larger CSF influx led to a more efficient glymphatic clearance, as illustrated by a rate of interstitial A β removal up to twice as fast in sleeping mice. Interestingly, the authors found that the volume of the interstitial space was over 60% greater when mice were sleeping or anesthetized, thus allowing easier CSF movement and, therefore, more efficient glymphatic clearance of neuronal by-products such as A β . Moreover, elevated A β levels as a consequence of sleep deprivation have been observed in CSF samples both in humans (Ooms et al., 2014) and in adult rats (Lihong Chen et al., 2017). One night of sleep deprivation also appeared to significantly increase PET A β plaque burden in the hippocampus and the thalamus in humans (Shokri-Kojori et al., 2018), although it is unclear how acute sleep deprivation may affect short-term plaque formation.

It seems that slow wave sleep (SWS; or ‘deep sleep’, dominated by large amplitude and low frequency EEG oscillations (Nir et al., 2011)) is a key component of the association between sleep-wake regulation and A β aggregation. The release of soluble A β in the interstitial space was shown to depend on endogenous neuronal activity, with extended wakefulness leading to an overall increase in neuronal firing and thus to elevated A β levels (Bero et al., 2011; Cirrito et al., 2005; Roh et al., 2012). In addition, slow wave activity (SWA; a quantification of sleep slow waves during sleep) in frontal regions was associated with higher CSF A β 42 levels in cognitively normal older individuals (Varga et al., 2016). Finally, a recent study showed that specific disruption of SWS during one night induced higher levels of CSF A β 40 on the following morning (Ju et al., 2017). Altogether, these results suggest that reduction of SWS may lead to a relative augmentation in neuronal activity during sleep that, in turn, increases the production of A β and/or reduces the effectiveness of its clearance. Recent evidence shows that A β concentration in the CSF increases following sleep deprivation while it does not decrease when attempting to enhance SWS, which may suggest that altered A β production, rather than its clearance, underlies the link between altered sleep and A β levels (Lucey et al., 2018). It remains to be determined whether the well-established modification in sleep structure in older people, including a significant reduction of SWS duration (Carrier et al., 2011; Mander et al., 2017), contributes to or is, in part, a consequence of the age-related increase in A β burden in healthy individuals (Braak, Thal, Ghebremedhin, & Tredici, 2011).

The depicted relationship between sleep-wake disruption and A β deposition is indeed most likely bidirectional (Cedernaes et al., 2017; Dufort-Gervais, Mongrain, & Brouillette, 2018; Ju, Lucey, & Holtzman, 2014). Albeit we emphasized that sleep-wake disorganization accelerates A β accumulation, it seems that A β deposits conversely impede sleep-wake regulation. A transgenic APP mouse model showed changes in the sleep-wake cycle that closely followed the emergence of A β plaques, and the intensity of these changes correlated with the extent of A β deposits (Roh et al., 2012). Interestingly, active A β immunization prevented these sleep-wake modifications, suggesting a direct impact of the presence of A β aggregates on sleep-wake regulation. Likewise, A β accumulation impaired slow waves propagation during sleep in AD mice whereas exogenous A β infusion disrupted SWA in wild-type mice until wash-out (Busche et al., 2015). In a *Drosophila* model of AD including human APP and beta-secretase cleaving enzyme expression, sleep is significantly disrupted when these proteins are co-expressed (Dissel et al., 2017).

Many other associations between sleep-wake regulation and A β are described below. We stress, however, that it is unclear whether these associations result from a causal impact of sleep-wake dysfunction or whether the latter may be a consequence of abnormal presence of A β . For instance, several studies showed that self-reported sleep quality is associated with A β deposition measured with PET and CSF markers in healthy older individuals (Branger et al., 2016; Spira et al., 2013; Sprecher et al., 2015, 2017). In two longitudinal studies, self-reported excessive daytime sleepiness at baseline was linked to increased accumulation of A β over 7 and 15.7 years in cognitively normal older individuals (Carvalho et al., 2018; Spira et al., 2018). Self-reported sleep latency was also associated with brain A β deposition in a well-characterized cohort of older men and women (Branger et al., 2016; B. M. Brown et al., 2016), while poor self-reported sleep history was associated with increased A β burden in the hippocampus and the thalamus (Shokri-Kojori et al., 2018). Objective measures of sleep efficiency, based on actigraphic recordings, were also linked to abnormal levels of amyloid in the CSF in healthy older adults (Ju et al., 2013). Likewise, an association was reported between PET A β pathology in the medial prefrontal cortex and slow waves generation (Mander et al., 2015). Obstructive sleep apnea (OSA), a well-established sleep disruption factor associated with a higher risk of developing cognitive impairments and AD (Andrade, Bubu, Varga, & Osorio, 2018), has been linked to increased CSF and PET A β burden in cognitively normal older adults (Elias et al., 2018; Sharma et al., 2018). One hypothesis is that glymphatic clearance processes are altered by mechanical changes during respiratory efforts in OSA patients, thus promoting protein accumulation (Andrade et al., 2018; Ju et al., 2016). Finally, rest-activity rhythm fragmentation, objectively estimated by actigraphic measures, has recently been associated with PET A β plaque burden, suggesting the implication of a circadian regulation of proteostasis (Musiek et al., 2018). This relationship remained significant after adjusting for age, implying that aging and preclinical A β pathology likely have separate contributions on sleep-wake disturbances.

Crucially, the interaction between worse sleep efficiency and abnormal CSF amyloid levels may account for worse memory function in the preclinical stages of AD (Molano, Roe, & Ju, 2017). This could mean that beyond the respective negative impact of poor sleep-wake quality (Yaffe, Falvey, & Hoang, 2014) and of A β burden (Bilgel et al., 2018) on cognitive function, there may be an interaction effect that would lead to a multiplicative impact of both, at least when exceeding a certain threshold. However, isolating their respective contributions

on cognition may constitute a difficult challenge since they are both changing importantly in aging.

The numerous findings reviewed here make a strong case for the bidirectional link between sleep-wake regulation and A β deposition. Interestingly, low-dose benzodiazepine administration to AD mice improved SWS and cognition, suggesting a potential role for γ -aminobutyric acid inhibition to alleviate part of A β negative impact through sleep (Busche et al., 2015). It is unknown, however, whether acting on A β *per se* and reducing its burden would result in an improved sleep-wake regulation. Likewise, no studies have yet attempted to act on sleep-wake quality to reduce A β burden or to slow down its increase.

Sleep-wake regulation and tau

Tau is a microtubule-associated protein whose primary role is to maintain the stability of the axonal cytoskeleton (Mietelska-Porowska, Wasik, Goras, Filipek, & Niewiadomska, 2014). In the course of several neurodegenerative diseases, including AD, tau undergoes abnormal hyperphosphorylation, oligomerization then conversion into insoluble filamentous state (Nelson et al., 2012; Spillantini & Goedert, 2013). Unable to interact with microtubules, hyperphosphorylated tau assembles into toxic oligomers which eventually form intracellular NFTs (Gómez-Ramos, Díaz-Hernández, Cuadros, Hernández, & Avila, 2006). Whether these NFTs are toxic or not remains debated (Spires-Jones, Kopeikina, Koffie, De Calignon, & Hyman, 2011), as they may not impede normal neuronal function once released in the extracellular space upon neuron death (Fox et al., 2011). As for A β , the AD-related pathophysiological process of tau follows a stereotyped progression pattern (Braak & Braak, 1991). As early as in the first decades of life, hyperphosphorylated tau can be found in the brainstem locus cœruleus and other subcortical nuclei (Braak & Del Tredici, 2011; Braak, Thal, Ghebremedhin, & Del Tredici, 2011), which are key sites in sleep-wake regulation (Saper, Scammell, & Lu, 2005). During the preclinical stages of AD, tau pathology is observed in the medial temporal lobe, and further spreads to the neocortex as the disease progresses (Braak, Alafuzoff, Arzberger, Kretschmar, & Tredici, 2006; Price et al., 2009; Price & Morris, 1999). Interestingly, abnormal levels of tau protein in the CSF in preclinical AD constitute an accurate proxy measure of tau deposition in the temporal lobe, which in turn is a strong predictor of subsequent cognitive trajectory (Brier, Gordon, Morris, Benzinger, & Ances, 2016).

Critically, Yamada et al. (Yamada et al., 2014) demonstrated using in vivo microdialysis in wild-type mice that increasing neuronal activity significantly elevated ISF tau levels within hours. Similar to A β regulation processes, this indicates that a relative increase in neuronal activity, as observed during extended wakefulness or chronic sleep restriction, could lead to an increase in extracellular tau production that would ultimately be reflected in CSF measures. However, tau is a relatively stable protein with a slow turnover rate, so that around 11 days are required before CSF changes can be observed (Price, Guan, Burlingame, Prusiner, & Ghaemmaghami, 2010; Yamada et al., 2014). This may explain why, contrary to A β , one night of acute sleep disruption could not be linked to an increase in CSF tau levels assessed on the subsequent morning in humans (Ju et al., 2017; Ooms et al., 2014). In fact, worse home sleep quality measured with actigraphic recordings during the week preceding in-lab sleep disruption was positively associated with higher CSF tau levels (Ju et al., 2017). In mouse models with plaques and tangles pathology, restricting sleep to 4 hours per day for 8 weeks (Di Meco, Joshi, & Praticò, 2014), or to 6 hours per day for 6 weeks (Rothman, Herdener, Frankola, Mughal, & Mattson, 2013), led to elevated insoluble tau levels. Accordingly in humans, a large longitudinal cohort study found that actigraphic measures of better sleep consolidation in older individuals significantly attenuated the effect of APOE genotype on observed NFTs density at autopsy (A. S. Lim, Yu, et al., 2013).

As for A β , the link between sleep-wake regulation and tau is likely bidirectional. A longitudinal study in cognitively normal older individuals showed that higher CSF tau levels were predictive of overall poorer sleep quality after 3 years (Fjell et al., 2017). However, this association was only present in individuals with significant A β deposition, further supporting the deleterious nature of tau and A β co-existence (Medeiros, Baglietto-Vargas, & Laferla, 2011). In addition, a human tau and amyloid knock-in mouse model of AD exhibits increased wake bout duration combined with decreased rapid eye movement (REM) and non-REM sleep duration (Jyoti, Plano, Riedel, & Platt, 2015; Platt et al., 2011). Transgenic mice expressing tau without A β abnormal accumulation display similar sleep-wake alterations, suggesting that tau pathology alone can induce impaired sleep and wakefulness in such animal models (Stevanovic et al., 2017). Of particular interest, the extent of the observed sleep impairments correlated with tau pathology intensity in brainstem regions regulating sleep (Holth, Mahan, Robinson, Rocha, & Holtzman, 2017). Likewise, progressive supranuclear palsy, a tau only form of frontotemporal dementia that mainly involves brainstem and thalamic regions, was associated with more fragmented sleep and increased daytime sleepiness (Arnulf et al., 2005;

Walsh et al., 2017), further suggesting a causal role for abnormal tau aggregation and sleep dysfunction.

Again similar to A β , many other associations between tau pathology and sleep-wake regulation have been reported, but it is difficult to identify the directionality of their interplay. In young and middle-aged individuals, OSA has been associated with elevated levels of tau protein concentration in blood (Bu et al., 2015; Motamedi et al., 2018). However, a recent study did not find any significant association between tau burden measured with PET and sleep disordered breathing in a cohort of 119 older males (Elias et al., 2018). In cognitively normal older people, CSF levels of phosphorylated tau were positively correlated with an increase in orexin concentration, potentially because elevated orexin levels favor fragmented sleep (Osorio et al., 2016) as previously observed in AD patients (Liguori et al., 2014; Slats, Claassen, Verbeek, & Overeem, 2013). Akin to A β , both objective and subjective measures of sleep quality (respectively, sleep efficiency derived from actigraphic recordings, and multidimensional self-reported scales) have been associated with elevated CSF tau levels (Ju et al., 2017; Sprecher et al., 2017). Finally, the fragmentation of the circadian rhythmicity of sleep and wakefulness positively correlates with the ratio between CSF phosphorylated tau and A β 42, a sensitive predictor of cognitive decline in nondemented older individuals (Fagan et al., 2007; Musiek et al., 2018).

Although convincingly demonstrated, the proofs of the bidirectionality of the link between tau pathology and sleep-wake regulation are less numerous than for A β . This probably resides in part in the absence of *in vivo* radiotracer for tau until very recently (Harada et al., 2015; Schöll et al., 2016), implying that one could only rely on CSF measure to infer tau burden *in vivo*. While the first PET markers suffered from relative unspecific bindings, the new generation seems to be particularly specific to tau (Villemagne, Doré, Burnham, Masters, & Rowe, 2018), such that our understanding of the association between tau and the sleep-wake cycle is likely to grow swiftly.

Critically, chronic sleep-wake disruption was consistently associated with tau phosphorylation changes and long-lasting memory deficits not only in AD mouse models, but also in wild-type littermates (Qiu et al., 2016). This suggests that recurrent sleep deprivation plays a significant role in tau-related measures as well as cognitive outcomes, beyond at least some genetic AD predispositions. Whether improving sleep-wake quality would reduce tau burden, or slow down its progression to then improve cognition, has however not been tested

yet. Likewise, no attempt has been made until now to address whether pharmacologically reducing tau burden would improve cognition via an enhancement of sleep-wake quality.

Sleep-wake regulation and neurodegeneration

Synaptic and neuronal loss represents one of the strongest pathological correlates of dementia (Jack et al., 2010; Terry et al., 1991), and a reliable predictor of conversion from MCI to AD (Bouwman et al., 2007; De Leon et al., 2004; Vemuri et al., 2009). Neurodegeneration of the medial temporal lobe can be detected up to 4 years before the clinical diagnosis of AD, and correlates with tau deposition as well as early memory deficits (Frisoni, Fox, Jack, Scheltens, & Thompson, 2010; Villemagne et al., 2013). The dynamic of brain atrophy mirrors the propagation of NFTs in early stages, then neuronal loss is progressively observed in temporo-parietal cortices, and ultimately in frontal regions (Thompson et al., 2003). In asymptomatic individuals, it was suggested that baseline rates of brain atrophy are accelerated in those that further transition to MCI (Fotenos, Snyder, Gitron, Morris, & Buckner, 2005). Although no magnetic resonance imaging (MRI) marker has been proved efficient to identify alone the preclinical stages of the disease, recent combinations of neuroimaging techniques hold strong potential for early detection of preclinical AD (Khan, 2018).

As for tau and A β burden, sleep-wake regulation is associated with neurodegeneration. In young adults, sleep continuity and sleep duration were associated with brain white matter integrity, based on measures of mean diffusivity derived from diffusion-weighted imaging (Takeuchi et al., 2018). In healthy older individuals, self-reported excessive daytime sleepiness and fatigue were associated to MRI measures of global and regional atrophy, as well as hippocampal volume reduction (Carvalho et al., 2017). Similarly, MRI based cortical thinning of several brain areas was linked to an age-related decrease in slow wave density and amplitude, based on electroencephalographic measures during sleep (Dubé et al., 2015). In addition, reduction of REM sleep duration in MCI subjects was associated with grey matter loss in regions that are affected early in AD, such as the precuneus, the posterior cingulate, and the postcentral gyrus (Sanchez-Espinosa, Atienza, & Cantero, 2014). Likewise, actigraphic assessment of sleep-wake rhythm fragmentation was related to the atrophy of the medial temporal lobe measured by expert visual evaluation (use of visual scale, i.e. no computerized quantification of MRI images) (Van Someren et al., 2018).

In cognitively normal older individuals, sleep durations shorter or longer than 7 hours have been associated with higher rates of frontotemporal grey matter decline in longitudinal assessments over 8 years (Spira et al., 2016). Others found that short sleep duration similarly affects the expansion rates of the ventricles (Lo, Loh, Zheng, Sim, & Chee, 2014). Moreover, self-reported sleep quality indices correlated with the rate of cortical atrophy measured over an average of 3.5 years, in a widespread set of frontal, temporal and parietal areas (Sexton, Storsve, Walhovd, Johansen-Berg, & Fjell, 2014). These longitudinal data suggest that sleep quality directly affects cortical atrophy. This view is further reinforced by the report of decreased neuron density in the locus coeruleus after extended wakefulness in wild-type mice (J. Zhang et al., 2014). Critically, chronic sleep restriction in mice lead to neuronal loss in the locus coeruleus that could not be compensated for after a 6-month recovery period in normal sleep conditions (Y. Zhu et al., 2007).

Normal aging is also associated with structural changes in sleep-wake regulating structures (Farajnia et al., 2014). The locus coeruleus shows a limited decline in neuron number with age and undergoes a marked reduction in the number of its projections to the cortex and particularly to the prefrontal cortex (Manaye et al., 1995; Mather & Harley, 2016). The basal forebrain, and especially the cholinergic neurons of the nucleus of Meynert (Nyakas et al., 2011), the suprachiasmatic nucleus corresponding to the master circadian clock (Palomba et al., 2008), or the lateral hypothalamus secreting orexin and melanin-concentrating hormone (Brownell & Conti, 2010), also undergo a decrease in neuron and/or axonal density in aging. These deficits are further aggravated in AD (Holth, Patel, & Holtzman, 2017; Satoh & Iijima, 2019; A. L. Stern & Naidoo, 2015). However, the functional consequences of the changes in sleep-wake regulating subcortical structures are mostly unassessed. It is therefore unclear whether they directly contribute to the well-characterized degradation in sleep quality and sleep-wake regulation associated with aging (Schmidt et al., 2012).

Hence, age-related neurodegeneration must undoubtedly contributes to changes in sleep and in wakefulness, but direct evidence remains scarce. How neurodegeneration contributes to AD through sleep remains therefore unclear. In turn, how sleep-wake dysfunction affects AD through neurodegeneration is not known. Addressing these issues is complicated notably because quantifying changes in brain structure *in vivo* is far from trivial. While the first demonstrations of age-related changes in MRI data were interpreted as indications of neurodegeneration, recent research showed that these changes may reflect modifications in neuronal iron or axonal myelin content rather than reflecting a mere loss of

neurons (Callaghan et al., 2014; C. Lambert, Chowdhury, et al., 2013). Crucially, the development of unbiased quantitative MRI (Weiskopf, Mohammadi, Lutti, & Callaghan, 2015) will provide important tools to address the link between sleep-wake quality and brain structure and microstructure.

Conclusions

We reviewed findings indicating that disruption of sleep-wake regulation is linked to the hallmarks of AD pathogenesis in the preclinical stages of the disease, that is, many years before the cognitive symptoms emerge. Evidence for bidirectional relationships between sleep-wake regulation and the three hallmarks of AD pathophysiology has accumulated, albeit more convincingly with A β . Recent technical developments in MRI and PET *in vivo* measures of brain structure will help establishing these relationships (Khan, 2018). Yet, research is still limited by the sensitivity of current techniques. Indeed, PET imaging does not allow to quantify the earliest tau (e.g. in the brainstem) or A β deposits. Besides, soluble forms of misfolded A β and tau proteins may be more involved in AD progression and neuronal death compared with their aggregates (Kopeikina, Hyman, & Spires-Jones, 2012; Masters & Selkoe, 2012). However, these soluble forms remain mostly undetectable *in vivo* and their impact on sleep-wake regulation is unknown. One could also consider that the locations of protein aggregates and of their soluble precursors is important, but this remains largely unexplored (see Mander et al. (Mander et al., 2015) for the importance of medial prefrontal A β deposits for SWS).

The large time-window of AD pathophysiological progression is a great opportunity for preventive interventions. However, it also means that curative or preventive interventions may be applied too late in the process to be efficient, i.e. when irreversible damage has occurred (Scheltens et al., 2016). Thus, the earliest aspects associated with AD pathogenesis need to be established. Factors related to sleep-wake regulation are, in our view, excellent candidates based on the findings reviewed here, but also given the fact that the first signs of tau deposition are detected, *post mortem*, in the locus cœruleus. However, these first deposits are undetectable *in vivo*, and their functional consequences are unknown (Mather & Harley, 2016). In addition, tau accumulation may primarily represents age-related modifications, i.e. brain changes that are normal over the lifespan (Duyckaerts et al., 2015). This does not preclude these protein accumulations, variable across individuals (Braak, Thal, Ghebremedhin, & Del Tredici, 2011), to contribute to the observed variability in age-related

changes in sleep-wake regulation, or to be caused by the latter variability. It is therefore highly needed, and yet highly challenging, to separate what constitutes normal neurodegeneration, protein accumulations, and sleep-wake changes from what will contribute to AD pathology decades later.

The definite proofs that improving sleep and wakefulness in the preclinical stages of the disease causally slows down the AD neuropathological processes over decades still need to be provided. Conversely, whether hindering tau and A β accumulation as well as neurodegeneration may improve sleep has to be demonstrated. Longitudinal studies in interventional designs are of particular interest to provide these proofs, but they may not be available before long. Even in the absence of such evidence, one could consider applying cognitive behavioral therapy (Morin, Vallières, & Ivers, 2009) or increasing the amplitude of the rest-activity cycle with light (Van Someren et al., 1999) and/or physical activity (P. Y. Yang, Ho, Chen, & Chien, 2012) to modulate AD neuropathological processes through improved sleep and wakefulness quality. Given the high prevalence of sleep-wake complaints in our 24-7 society, these appear as useful examples of reasonable and easy strategies to improve brain function in the short term, and to decrease the odds of developing AD, particularly in those individuals that are more at risk.

Table 1. Summary table of studies considered in this review and directly linking sleep-wake regulation to amyloid-beta.

Reference	Target population (N, age)	Sleep-wake variable(s)	A β variable(s)	Main outcome(s)
Branger et al., 2016	Cognitively normal participants (51, 64.1 $\pm$ 10.6 years)	Self-reported sleep latency and sleep quality	Cortex A β burden	Longer sleep latency and poorer sleep quality were associated with increased A β burden.
Brown et al., 2016	Cognitively normal participants (184, 75.5 $\pm$ 6.1 years)	Sleep latency	Cortex A β burden	Longer sleep latency was associated with increased A β burden.
Busche et al., 2015	APP23xPS45 mice (5, 6-8 months), wild-type mice (6, 6-8 months)	Long-range SWA coherence	Cortex, hippocampus, and thalamus A β burden, exogenous A β infusion	SWA coherence between cortex and hippocampus was disrupted in transgenic mice. A β administration was associated with impaired SWA coherence in wild-type mice.
Carvalho et al., 2018	Cognitively normal participants (283, 77.1 $\pm$ 4.8 years)	ESS	Longitudinal A β accumulation	Excessive daytime sleepiness was associated with increased A β accumulation over 7 years of follow-up.
Chen et al., 2017	Male Sprague-Dawley rats (40, 3 months)	2-4 days of paradoxical sleep deprivation	Hippocampus A β burden	Acute sleep deprivation was associated with increased A β burden

Dissel et al., 2017	Adult UAS-APP:BACE flies (30, 7 days)	Sleep latency, sleep fragmentation, sleep duration	Human APP and BACE co-expression	Co-expression of APP and BACE was associated with reduced sleep duration and increased sleep fragmentation.
Elias et al., 2018	Male cognitively normal OSA patients (42, 67.69 ± 5.37 years) and controls (77, 68.3 ± 3.86 years)	Apnea-hypopnea index	Cortex Aβ burden	Aβ burden was increased in OSA patients compared to controls.
Ju et al., 2013	Cognitively normal participants (142, 65.6 ± 8.2 years)	2 weeks of at-home actigraphic recording	CSF-Aβ level	Worse sleep efficiency was associated with abnormal CSF-Aβ level.
Ju et al., 2016	Cognitively normal OSA patients (10, 48-62) and controls (31, 45.8-65.7 years)	Apnea-hypopnea index, NREM SWA	CSF-Aβ level	Reduced NREM SWA was associated with increased CSF-Aβ level in controls. Higher apnea-hypopnea index was associated with abnormal CSF-Aβ level.
Ju et al., 2017	Cognitively normal participants (14, 35-65 years)	1 night of specific SWA disruption	CSF-Aβ level	Specific SWA disruption was associated with higher CSF-Aβ level.

Kang et al., 2009	Tg2576 mice (16, 3-9.5 months), C57BL6/SJL mice (20, 4 months), APP ^{SWE} /PS1 ^{ΔE9} mice (38, 1.7-3.5 months) Male cognitively normal participants (10, 20-50 years)	Acute sleep deprivation, sleep restriction to 4h/day for 3 weeks, almorexant injection (1/day for 8 weeks)	ISF-Aβ level, CSF-Aβ level, cortex and hippocampus Aβ burden	ISF- and CSF-Aβ levels showed fluctuations over a 24h-period. Acute and chronic sleep deprivation were associated with increased ISF-Aβ level and increased Aβ burden, respectively. Chronic almorexant injection was associated with reduced Aβ burden.
Lucey et al., 2018	Cognitively normal participants (8, 30-60 years)	1 night of sleep deprivation, 1 night of SWS augmentation	CSF-Aβ level	Total sleep deprivation was associated with increased CSF-Aβ level. No association between SWS augmentation and CSF-Aβ level.
Mander et al., 2015	Cognitively normal participants (26, 75.1 ± 8.2 years)	NREM SWA	Medial prefrontal cortex Aβ burden	Increased Aβ burden was associated with impaired NREM SWA.
Molano et al., 2017	Cognitively normal participants (98, 69 ± 7.1 years)	2 weeks of at-home actigraphic recording	CSF-Aβ level	The interaction between worse sleep efficiency and abnormal CSF-Aβ level was associated with poorer cognition.

Musiek et al., 2018	Cognitively normal participants (148, 66.6 ± 8.3 years)	1 week of at-home actigraphy	Cortex Aβ burden	Higher fragmentation of rest-activity circadian rhythm was associated with increased Aβ burden.
Ooms et al., 2014	Male cognitively normal participants (26, 40-60 years)	1 night of total sleep deprivation	CSF-Aβ level	Total sleep deprivation was associated with increased CSF-Aβ level.
Roh et al., 2012	APP ^{SWE} /PS1 ^{ΔE9} mice (32, 3-9 months)	Wakefulness duration, NREM/REM sleep duration	Hippocampus Aβ burden, active immunization to Aβ	Increased Aβ burden was associated with increased wakefulness duration and decreased NREM/REM duration. Active immunization to Aβ restored the sleep-wake cycle.
Roh et al., 2014	APP ^{SWE} /PS1 ^{ΔE9} /OR ^{-/-} mice (12, 3-6 months), APP/PS1-21/OR ^{-/-} mice (12, 3.5-8.5 months)	Orexin modulation, sleep restriction to 4h/day for 3 weeks	Cortex and hippocampus Aβ burden	Transgenic orexin knockout mice displayed decreased Aβ burden and increased sleep duration. Chronic sleep restriction was associated with increased Aβ burden.

Sharma et al., 2018	Cognitively normal OSA patients (111, 69.26 ± 7.41 years) and controls (97, 67.56 ± 7.32 years)	Apnea-hypopnea index	CSF-A β level, longitudinal A β accumulation	Higher apnea-hypopnea index was associated with abnormal CSF-A β level and increased A β accumulation over 2 years of follow-up.
Shokri-Kojori et al., 2017	Cognitively normal participants (24, 22-72 years)	1 night of total sleep deprivation, self-reported sleep history	Hippocampus and thalamus A β burden	Total sleep deprivation and poorer self-reported sleep history were associated with increased A β burden.
Spira et al., 2013	Cognitively normal participants (70, 53-91 years)	Self-reported sleep duration and sleep quality	Cortex and precuneus A β burden	Poorer sleep quality and shorter sleep duration were associated with increased A β burden.
Spira et al., 2018	Cognitively normal participants (123, 36.2-82.7 years)	Self-reported excessive daytime sleepiness and napping habits	Longitudinal A β accumulation	Excessive daytime sleepiness was associated with increased A β burden over 15.7 years follow-up. No association between napping and A β burden.
Sprecher et al., 2015	Cognitively normal participants (98, 50-73 years)	ESS, MOS	Cortex A β burden	Greater somnolence and sleep disturbances were associated with increased A β burden.

Sprecher et al., 2017	Cognitively normal participants (101, 62.9 ± 6.2 years)	MOS, ESS	CSF-A β level	Poorer sleep quality, greater sleep disturbance and daytime somnolence were associated with abnormal CSF-A β level.
Varga et al., 2016	Cognitively normal participants (36, 66.8 ± 8.2 years)	SWA, SWS duration	CSF-A β level	Reduced SWA and SWS duration were associated with higher CSF-A β level.
Xie et al., 2013	C57BL6 mice (12, 3 months)	Natural and induced sleep	A β glymphatic clearance rate	Natural and induced sleep were associated with a 60% increase in interstitial space volume, resulting in easier glymphatic clearance.

Abbreviations: A β = Amyloid-beta; APP = amyloid precursor protein; BACE = Beta-secretase cleaving enzyme; CSF = Cerebrospinal fluid; ESS = Epworth sleepiness scale; ISF = Interstitial fluid; MOS = Medical outcomes study sleep scale; NREM = Non-rapid eye movement; OSA = Obstructive sleep apnea; REM = Rapid eye movement; SWA = Slow wave activity; SWS = Slow wave sleep.

Table 2. Summary table of studies considered in this review and directly linking sleep-wake regulation to tau.

Reference	Target population (N, age)	Sleep-wake variable(s)	Tau variable(s)	Main outcome(s)
Arnulf et al., 2005	PSP patients (15, 68 ± 8 years) and cognitively normal controls (15, 67 ± 10 years)	Sleep fragmentation, REM sleep without atonia, RBD	Brain tau accumulation without abnormal Aβ burden	Higher sleep fragmentation, longer REM sleep without atonia duration, and RBD in PSP patients compared to controls.
Bu et al., 2015	Cognitively normal OSA patients (45, 44.31 ± 9.96 years)	Apnea-hypopnea index	Serum tau level	Higher apnea-hypopnea index was associated with higher serum tau level.
Di Meco et al., 2014	Male 3xTG mice (18, 10 months)	Sleep restriction to 4h/day for 8 weeks	Brain insoluble tau burden	Chronic sleep restriction was associated with increased insoluble tau level.
Elias et al., 2018	Male cognitively normal OSA patients (42, 67.69 ± 5.37 years) and controls (77, 68.3 ± 3.86 years)	Apnea-hypopnea index	Cortex tau burden	No difference in tau burden in OSA patients compared to controls.
Fjell et al., 2017	Cognitively normal participants (91, 64-89 years)	PSQI	CSF-tau level	Higher CSF-tau level was predictive of worse sleep quality in Aβ positive individuals.

Holth et al., 2017	Male P301S mice (40, 3-12 months)	Wake bout duration, NREM/REM sleep duration	Brainstem tau burden	Higher brainstem tau burden was associated with increased wakefulness, decreased NREM and REM sleep duration.
Ju et al., 2017	Cognitively normal participants (14, 35-65 years)	1 week of at-home actigraphic recording, 1 night of specific SWA disruption	CSF-tau level	No association between SWA disruption and CSF-tau level. Better home sleep quality was associated with lower CSF-tau level.
Jyoti et al., 2015	PLB1 _{triple} mice (14, 5-21 months)	Wake bout duration, NREM/REM sleep duration	Cortex and hippocampus tau burden	Tau burden was associated with increased wakefulness, and reduced NREM and REM sleep duration.
Lim et al., 2013	Cognitively normal participants (201, 85.9 ± 6.3 years)	10 days of actigraphic recordings	Brain NFTs density at autopsy	Better sleep consolidation was associated with decreased NFTs density.
Motamedi et al., 2017	Cognitively normal OSA patients (50, 34.9 ± 8 years)	Apnea-hypopnea index	Plasma tau level	Higher apnea-hypopnea index was associated with higher plasma tau level.
Musiek et al., 2018	Cognitively normal participants (148, 66.6 ± 8.3)	1 week of at home actigraphy	CSF-tau level	Higher fragmentation of rest-activity circadian rhythm was associated with higher CSF-tau level.

Ooms et al., 2014	Cognitively normal men (26, 40-60 years)	1 night of total sleep deprivation	CSF-tau level	No association between total sleep deprivation and CSF-tau level.
Osorio et al., 2016	Cognitively normal participants (63, 69.59 ± 8.55 years)	CSF-orexin level	CSF-tau levels	Higher CSF-orexin level was associated with higher CSF-tau levels.
Platt et al., 2011	PLB1 ^{triple} mice (11, 5-12 months)	Wake bout duration, NREM sleep duration	Cortex and hippocampus tau burden	Tau burden was associated with increased wakefulness and reduced NREM sleep duration.
Qiu et al., 2016	APP ^{SWE} /PS1 ^{ΔE9} mice (40, 4-10 months), wild-type littermates (40, 4-10 months)	Sleep restriction to 4h/day for 8 weeks	Frontal cortex and hippocampus tau burden	Chronic sleep deprivation was associated with long-lasting increased tau burden in both transgenic and wild-type mice.
Rothman et al., 2013	Male 3xTG mice (10, 14 months)	Sleep restriction to 6h/day for 6 weeks	Cortex and hippocampus tau burden	Chronic sleep restriction was associated with increased cortical tau burden.
Sprecher et al., 2017	Cognitively normal participants (101, 62.9 ± 6.2 years)	MOS, ESS	CSF-tau level	Higher excessive daytime sleepiness and worse subjective sleep quality were associated with higher CSF-tau level.

Stevanovic et al., 2017	Tg4510 mice (11, 8-13 months)	Rest-activity circadian rhythm, clock gene (<i>BMAL1</i> , <i>PER2</i>) expression	SCN and hippocampus tau burden	Tau burden was associated with altered SCN circadian outputs and clock protein (<i>PER2</i>) rhythm in the hippocampus.
Walsh et al., 2017	PSP patients (19, 70.94 ± 5.3 years) and cognitively normal controls (16, 72.50 ± 1 years)	Sleep latency, sleep duration, sleep fragmentation, subjective sleepiness	Brain tau accumulation without abnormal Aβ burden	Longer sleep latency, lower sleep duration, higher sleep fragmentation, and higher subjective sleepiness were found in PSP patients compared to controls.

Abbreviations: Aβ = Amyloid-beta; CSF = Cerebrospinal fluid; ESS = Epworth sleepiness scale; MOS = Medical outcomes study sleep scale; NFTs = Neurofibrillary tangles; NREM = Non-rapid eye movement; OSA = Obstructive sleep apnea; PSQI = Pittsburgh sleep quality index; RBD = Rapid eye movement sleep behavior disorder; REM = Rapid eye movement; SCN = Suprachiasmatic nucleus; SWA = Slow wave activity.

Table 3. Summary table of studies considered in this review and directly linking sleep-wake regulation to neurodegeneration.

Reference	Target population (N, age)	Sleep-wake variable(s)	Neurodegeneration variable(s)	Main outcome(s)
Carvalho et al., 2017	Cognitively normal participants (1374, 72.16 ± 8.8 years)	ESS, fatigue	Grey matter integrity	Higher excessive daytime sleepiness and fatigue were associated with lower cortical thickness.
Dubé et al., 2015	Cognitively normal participants (63, 20-70 years)	Sleep slow waves density and amplitude	Grey matter integrity	Higher sleep slow waves density and amplitude were associated with higher grey matter integrity in sleep slow waves related regions.
Lo et al., 2014	Cognitively normal participants (66, 69.5 ± 5.7 years)	Self-reported sleep duration	Longitudinal ventricles expansion rate	Reduced sleep duration was associated with faster annual expansion rates of the ventricles over 2 years of follow-up.
Sanchez-Espinosa et al., 2014	aMCI participants (21, 69.8 ± 5.5 years)	REM sleep duration	Grey matter integrity	Lower REM sleep duration was associated with reduced grey matter integrity in brain regions involved in early stages of AD.

Sexton et al., 2014	Cognitively normal participants (147, 20.4-84.2 years)	PSQI	Longitudinal grey matter decline	Worse sleep quality was associated with higher rates of grey matter atrophy over 3.5 years of follow-up.
Spira et al., 2016	Cognitively normal participants (122, 51-86 years)	Self-reported sleep duration	Longitudinal grey matter decline	Sleep durations of less or more than 7 hours were associated with higher rates of grey matter atrophy over 8 years of follow-up.
Takeuchi et al., 2018	Cognitively normal participants (1201, 18-27 years)	Self-reported sleep continuity and sleep duration	White matter integrity	Higher sleep continuity and lower sleep duration were associated with higher white matter integrity.
Van Someren et al., 2018	Cognitively normal participants (138, 69.1 ± 8.5 years)	1 week of at home actigraphic recording	Medial temporal lobe atrophy	Higher fragmentation of rest-activity circadian rhythm was associated with higher medial temporal lobe atrophy.
Zhang et al., 2014	Male SirT3 wild-type mice (5, 2 months)	Sleep restriction to 4h/day	Number of locus coeruleus neurons	Extended wakefulness was associated with a loss of neurons in the locus coeruleus.

Zhu et al., 2007	Male C57BL/6J mice (10, 2 months)	Long-term intermittent hypoxia exposure for 8 weeks, sleep duration, sleep latency	Number of locus coeruleus neurons.	Chronic sleep disruption was associated with a loss of neurons in the locus coeruleus that had long-lasting effects on sleep duration and sleep latency.
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Abbreviations: aMCI = Amnestic mild cognitive impairment; ESS = Epworth sleepiness scale; PSQI = Pittsburgh sleep quality index; REM = Rapid eye movement.

Sleep and wakefulness optimization

In non-pathological contexts, wakefulness is a time of constant experience of the environment, be it through sensory reactions, motor behaviors, associative processing, thinking, etc. (Tononi & Cirelli, 2003). More than just being conscious, the awake state is prone to interactions with external stimuli, supporting crucial learning and behavioral adjustments to adapt to the outside world. At the brain level, this constant wake-dependent flow of information is integrated through neuroplasticity within neuronal networks: several studies, both in animals and in humans, have shown that sustained wakefulness is associated with synaptic plasticity changes and potentiation resulting in a net increase in synaptic weight (Bernardi et al., 2015; Bushey, Tononi, & Cirelli, 2011; De Vivo et al., 2017; Vyazovskiy, Cirelli, Pfister-Genskow, Faraguna, & Tononi, 2008; Vyazovskiy & Faraguna, 2015). Importantly, the wake-dependent increase in synaptic strength has been shown to depend on the content of wakefulness, that is, cortical circuits that are recruited more when experiencing the environment display a local and use-dependent increase in synaptic potentiation (Bernardi et al., 2015; Hung et al., 2013; Kuhn et al., 2016; Rao et al., 2007; Siclari & Tononi, 2017). In addition, the progressive increase in synaptic weight is inevitably accompanied by higher resources demands at the cellular level, as reflected by higher energy consumption and increased need in cellular supplies (Attwell & Gibb, 2005; Bellesi, de Vivo, Koebe, Tononi, & Cirelli, 2018; Tononi & Cirelli, 2014). Considering this relationship between wakefulness, synaptic plasticity, and cellular costs, a regulatory mechanism is therefore needed to restore synapses to their baseline condition and enable potential new learning; such role may be endorsed by sleep. This rationale has led to the development of the synaptic homeostasis hypothesis (SHY), whose general postulate is that sleep is the price for plasticity (Tononi & Cirelli, 2003, 2006, 2014).

According to this view (**Figure 7**), the intensity of prior wakefulness (*i.e.* the amount of wake- and task-dependent synaptic strengthening) will both locally and globally determine EEG characteristics during the following sleep period, which is in turn considered as a necessary off-state to ‘reset’ synapses in order to optimize the subsequent period of wakefulness. Particularly, the homeostatic marker of NREM SWA would play a crucial role in the sleep-wake regulation of synaptic balance. Indeed, studies highlighting the local effect of wakefulness on synaptic strength have also demonstrated that, compared to other brain areas, the recruited task-dependent cortical circuits generate significantly more slow waves in the subsequent sleep period (Bernardi et al., 2015; Cottone, Adamo, & Squires, 2004; Huber

et al., 2004; Hung et al., 2013; Kuhn et al., 2016; Miyamoto, Katagiri, & Hensch, 2003; Vyazovskiy, Borbély, & Tobler, 2000). Sleep is thought to restore cellular needs by favoring glucose storage (Bellesi et al., 2018), potentially through the association between slow waves generation and increased cerebral blood flow as well as larger extracellular space (Fultz et al., 2019). In addition, NREM SWA would constitute a suitable ground for overall down-selection/depotentialization of synapses to baseline levels in order to achieve synaptic homeostasis, and the typical decreasing time course of slow waves across a night of sleep would reflect the underlying depotentialization process (Tononi & Cirelli, 2003). This ‘down-selection’, mediated by synaptic depression-inducing proteins such as Arc (El-Boustani et al., 2018) or Homer1a (Diering et al., 2017) would result in an increased signal-to-noise ratio, leading to the consolidation and integration of information acquired during wakefulness (Tononi & Cirelli, 2019).

It is important to mention that, although encompassing a number of unique aspects in a seemingly elegant way, SHY still has several unanswered questions and the validity of its core concepts is an ongoing debate in the field (Cirelli & Tononi, 2019; Frank, 2013; Frank & Seibt, 2018; Siclari & Tononi, 2017; Tononi & Cirelli, 2019). Of central importance, SHY has difficulties to provide clear mechanistic pathways of its main tenet, and cannot reconcile with empirical data showing that sleep can promote the formation of new synapses and their strengthening (Frank, 2012; Raven, Van der Zee, Meerlo, & Havekes, 2018).

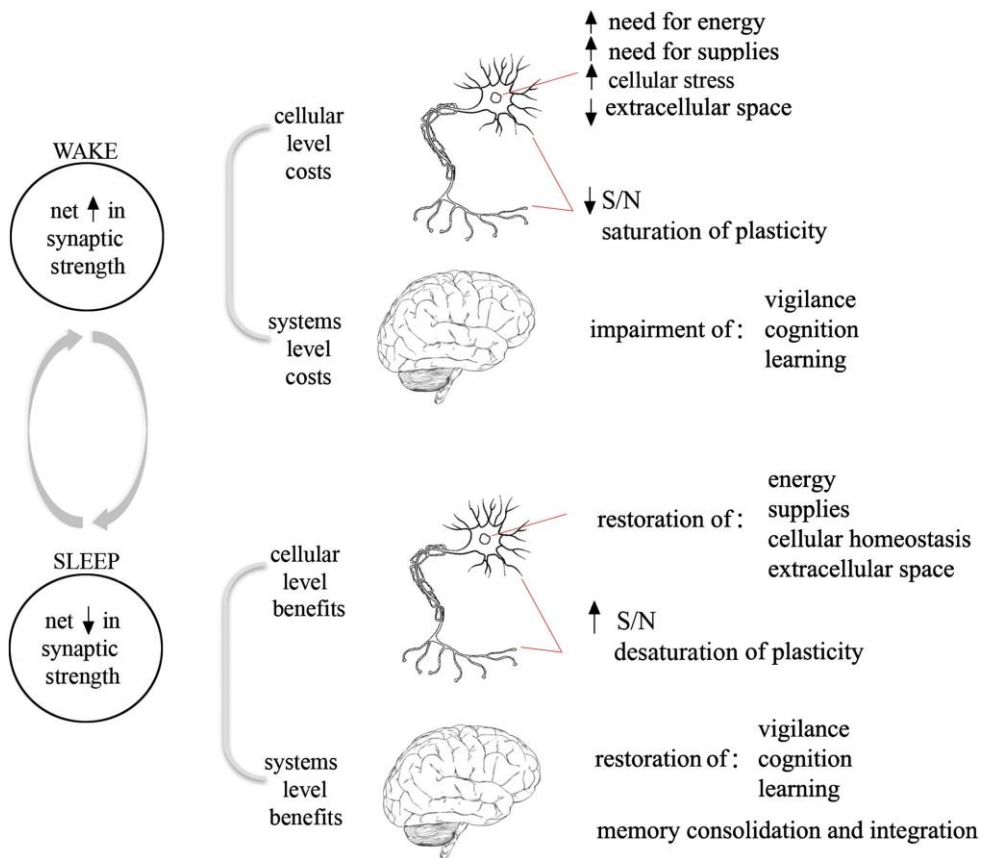


Figure 7. The synaptic homeostasis hypothesis. Summary illustration of the wake- and sleep-dependent cellular processes underlying the regulation of synaptic strength across the sleep-wake cycle. S/N: signal-to-noise ratio. From (Tononi & Cirelli, 2014).

Experimental protocol

The COFITAGE study

All results presented in this thesis are based on data from a large cross-sectional multimodal study carried out at the GIGA-Cyclotron Research Centre, Liège, starting from June 2016 until October 2019. The core research framework of the ‘Cognitive Fitness in Aging’ (COFITAGE) study is deeply-rooted in the search for new markers of so-called ‘successful cognitive aging’ (Daffner, 2010). Its general aim is to characterize, in a sample of 101 healthy older individuals aged 50-70 years, the role of sleep-wake regulation in the early pathogenesis of AD and its relationships with cognitive trajectory, while also investigating the function of potential reserve factors such as allostatic load, cognitive and brain reserve, and genetic background. Since AD features a large time window of progressive neuropathophysiological changes prior to the onset of cognitive symptoms (Jack et al., 2013; Sperling et al., 2011; Sperling, Mormino, & Johnson, 2014), the COFITAGE study was developed to unravel new early (bio-)markers associated with current cognitive fitness and well-established AD biomarkers, in order to help predict subsequent cognitive decline and guide new potential therapeutic interventions (**Figure 8**).

In the context of the present thesis, the specific methods used for the three reported set of findings is described in more detailed in their respective results section. Each of these analyses includes samples with different numbers of subjects depending on data availability at the time. The first set is based on data from 60 subjects, the second included 65 subjects, and the last involves data from the final 101 subjects. For the sake of clarity and to be exhaustive, an overview of the whole COFITAGE study is provided in **Figure 9**.

In brief, participants included in the experimental protocol underwent the following assessments (not in order): 1) Repeated TMS-EEG, resting-state wakefulness EEG, cognitive, and pupillometric recordings during 20h of wakefulness extension in constant routine conditions; 2) One night of regular sleep under EEG; 3) [¹⁸F]Flutemetamol and [¹⁸F]THK-5351 PET scans to quantify amyloid-beta and tau proteins burden, respectively; 4) Quantitative multi-parametric and diffusion-weighted MRI; 5) Two weeks of free sleep-wake timings and one week of fixed sleep-wake schedule assessed via actimetry; 6) Collection of blood and 24-h urinary samples to compute allostatic load and to get genetic information; 7) Questionnaires of lifespan habits underlying cognitive reserve; 8) Extensive cross-sectional neuropsychological assessments of memory, attention, and executive functions; 9) Longitudinal assessment of these cognitive functions after 2 years.

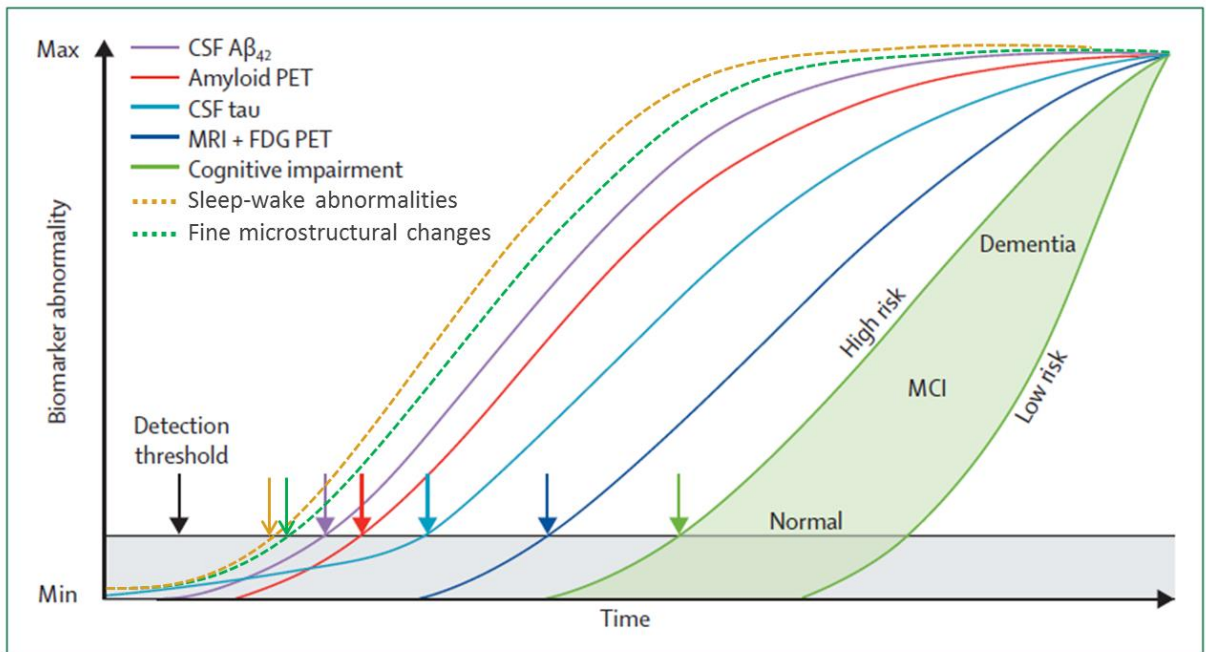


Figure 8. Visual representation of AD preclinical time window. Preclinical AD is characterized by neuropathophysiological changes, such as abnormal levels of amyloid-beta and tau proteins in the CSF, that precede the onset of cognitive symptoms (mild cognitive impairment, MCI) and are progressively observable along disease progression towards dementia. The main objectives of the COFITAGE study are summarized and illustrated by the yellow and green dotted lines: abnormalities in sleep-wake regulation and/or fine microstructural changes may constitute detectable early (and potentially earlier) markers of AD-related alterations and possibly predict subsequent cognitive trajectory. Modified from (Jack et al., 2013)

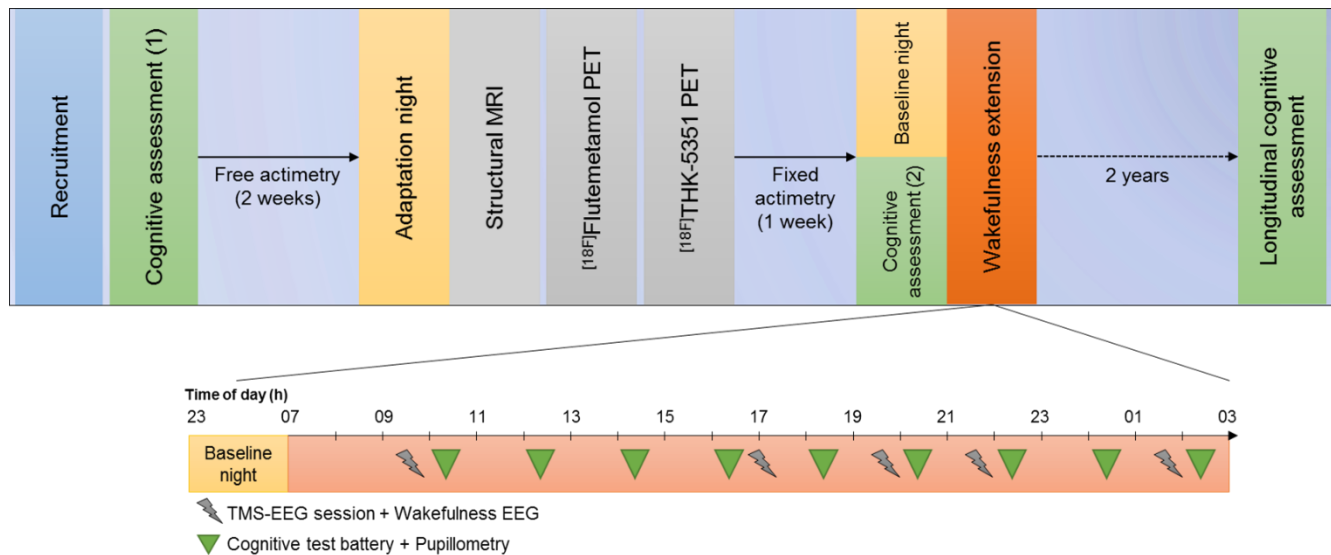


Figure 9. Overview of the whole COFITAGE study. Schematic representation of the study design for a prototypical participant with bedtime = 23h and wake-up time = 07h. The adaptation night of sleep under polysomnography also served as a screening for sleep apneas. Collection of blood and 24-h urinary samples were performed after the adaptation night and during the wakefulness extension protocol, respectively. Demographic and cognitive reserve questionnaires were administered during recruitment and wakefulness extension protocol. Schedules for fixed actimetry, baseline night, and wakefulness extension were based on participants' habitual bedtime and wake-up time. The extensive cross-sectional neuropsychological assessment was split into two parts and administered while participants were well rested. The order of the different parts of the protocol was subject to slight changes, except for the period starting with fixed actimetry and ending with wakefulness extension. Of note, the follow-up cognitive assessment aiming at investigating cognitive decline after a 24-month period is still ongoing.

Experimental results

Chapter I

Preserved wake-dependent cortical excitability dynamics predict cognitive fitness beyond age-related brain alterations

This results section has been accepted for publication in *Communications Biology* as a Research Article. Supplementary materials related to this manuscript are displayed in **Appendix 1**.

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Abstract

Age-related cognitive decline arises from alterations in brain structure as well as in sleep-wake regulation. Here, we investigated whether preserved wake-dependent regulation of cortical function could represent a positive factor for cognitive fitness in aging. We quantified cortical excitability dynamics during prolonged wakefulness as a sensitive marker of age-related alteration in sleep-wake regulation in 60 healthy older individuals (50-69y; 42 women). Brain structural integrity was assessed with amyloid-beta- and tau-PET, and with MRI. Participants' cognition was investigated using an extensive neuropsychological task battery. We show that individuals with preserved wake-dependent cortical excitability dynamics exhibit better cognitive performance, particularly in the executive domain which is essential to successful cognitive aging. Critically, this association remained significant after accounting for brain structural integrity measures. Preserved dynamics of basic brain function during wakefulness could therefore be essential to cognitive fitness in aging, independently from age-related brain structural modifications that can ultimately lead to dementia.

Introduction

Aging is associated with an overall cognitive decline triggered in part by a progressive degradation of brain structure. Limited but notable neuronal loss takes place during healthy adulthood (Giorgio et al., 2010). In addition, tau protein, which stabilizes axonal structure and contributes to synaptic function, and amyloid-beta ($A\beta$) protein, a peptide directly related to neuronal activity, progressively aggregate in the brain over the lifespan to form neurofibrillary tangles (NFTs) and senile plaques, respectively (Jack et al., 2013). Tau NFTs, $A\beta$ plaques, and neurodegeneration favor cognitive decline (Reuter-Lorenz & Park, 2014). They are considered as major underlying causes of dementia and constitute the hallmarks of Alzheimer's disease (AD) (Jack et al., 2018). However, age-related changes in brain structure go undetected for decades: tau protein aggregation takes place as early as during the second decade of life in the brainstem, while $A\beta$ aggregates can be detected around the 4th decade in the neocortex (Braak, Thal, Ghebremedhin, & Tredici, 2011).

One of the first signs of AD- and age-related brain structural degradation may reside in alterations in the regulation of sleep and wakefulness (Van Egroo, Narbutas, Chylinski, Villar González, Maquet, Salmon, Collette, et al., 2019). Sleep-wake disruption is indeed strongly associated with AD neuropathology (Van Someren et al., 2018): grey matter (GM) integrity has been associated with measures of sleep quality, including sleep slow waves characteristics, in cross-sectional and longitudinal studies (Dubé et al., 2015; Sexton et al., 2014). $A\beta$ and tau burdens in healthy older individuals have been associated with the amount of slow waves generated during non-rapid eye movement (NREM) sleep (Lucey et al., 2019; Mander et al., 2015). Importantly, $A\beta$ burden has been reported to affect memory performance through its impact on sleep slow waves in elderly individuals (~75 y) (Mander et al., 2015). In addition, the presence of preclinical $A\beta$ plaque pathology, assessed through PET imaging or cerebrospinal fluid collection, is associated with fragmentation of the entire rest-activity cycle, *i.e.* encompassing both sleep and wakefulness (Musiek et al., 2018). Whether age-related alteration in brain structure may affect sleep-wake regulation of daytime brain activity is unknown, however.

Sleep and wakefulness are regulated by two fundamental processes: sleep homeostasis, which keeps track of time awake, and circadian rhythmicity, which temporally organizes physiology and behavior (Duffy & Dijk, 2002; Schmidt et al., 2012). The strength of both processes seems to decrease with age, resulting in dampened dynamics of sleep-wake

rhythms and reduced variations in brain activity both during sleep and prolonged wakefulness (Schmidt et al., 2012). The generation of slow waves during sleep, which is associated with the dissipation of sleep need, is reduced in aging (Carrier et al., 2011). Likewise, cortical excitability (Gaggioni et al., 2019), a basic aspect of brain function implicated in age-related cognitive decline (Oh, Oliveira, & Disterhoft, 2010; Rizzo, Richman, & Puthanveetil, 2015), shows less variations during prolonged wakefulness. Age-related alterations in the regulation of sleep and wakefulness are not only associated with current cognition (Oosterman, Van Someren, Vogels, Van Harten, & Scherder, 2009; Yaffe et al., 2014), but also predicts future cognitive trajectories, including the risk of developing dementia (Bubu et al., 2017; A. S. Lim, Kowgier, et al., 2013; Musiek et al., 2018; Shi et al., 2018). Importantly, some of the changes in sleep-wake regulation take place as early as in middle-aged individuals (> 40y) (Carrier et al., 2011). Whether the early alterations in sleep and wakefulness regulation and their potential cognitive consequences are systematically related to age-related alterations in brain structure remains unknown.

The goals of the present study were threefold. First, we assessed whether sleep-wake regulation of the awake and active brain is linked to age-related alterations in brain structure in healthy older individuals (50-70 years). We measured cortical excitability based on electroencephalographic (EEG) responses to transcranial magnetic stimulation (TMS) during a wake-extension protocol and hypothesized that the dynamics of cortical excitability during wakefulness would be related to both A β and tau burden, taking into account any potential neurodegeneration. We further investigated whether wake-dependent variations in cortical excitability are linked to cognitive fitness. Based on previous findings (Gaggioni et al., 2019), we anticipated that cortical excitability dynamics would be associated with executive performance. Finally, we tested whether these putative links would be independent of A β and tau burden as well as neurodegeneration. We postulated that the inclusion of the three markers of brain structural integrity in our statistical models would at least decrease, if not remove, the association between cognition and wake-dependent cortical excitability dynamics.

Analyses reveal that frontal cortical excitability dynamics during prolonged wakefulness display a high variability across individuals, with some older people exhibiting preserved or young-like regulation profiles of cortical function. Preserved cortical excitability regulation profiles were associated with better cognitive performance, particularly in the executive domain which is central to successful cognitive aging. Importantly, the association

between cognition and wake-dependent regulation of cortical function was independent of grey matter volume as well as A β and tau protein burden.

Results

In a multi-modal cross-sectional study (**Fig. I.1A**), 60 healthy and cognitively normal late middle-aged individuals (42 women; age range 50-69 years, mean $\pm$ SD = 59.6 $\pm$ 5.5 years; **Table I.1**) underwent structural MRI to measure GM volume, as well as [^{18}F]Flutemetamol and [^{18}F]THK-5351 PET imaging to quantify A β and tau burden, respectively. Participants' cognitive performance while well-rested was assessed with an extensive neuropsychological task battery probing memory, attention, and executive functions. After a week of regular sleep-wake schedule, participants' habitual sleep was recorded in-lab under EEG to quantify slow waves generation during NREM sleep. A wake-extension protocol started on the following day and consisted of 20h of continuous wakefulness to trigger a moderate realistic sleep-wake challenge. Wake-extension was conducted under strictly controlled constant routine conditions known to unmask the combined influences of sleep homeostasis and of the circadian system by removing or reducing potential biases from light exposure, physical activity, food intake or room temperature (Duffy & Dijk, 2002). Cortical excitability over the frontal cortex was measured 5 times over the 20h protocol, using transcranial magnetic stimulation combined with an electroencephalogram (TMS-EEG) (**Fig. I.1B-D**) (Gaggioni et al., 2019; Huber et al., 2013). Mean time between wake-extension protocol and cognitive assessment was 30.6 $\pm$ 37.7 days. Mean time between wake-extension protocol and brain structural integrity assessments was 56.7 $\pm$ 78.9 days for MRI, 121.3 $\pm$ 97.1 days for A β -PET, and 116.3 $\pm$ 108.3 days for tau-PET.

Whole-brain GM volume was 41.04 $\pm$ 3.73% of total intracranial volume and showed the expected reduction with increasing age ($F_{1,56} = 5.86$, $p = 0.02$, semi-partial R^2 ($R^2_{\beta^*}$) = 0.10; **Supplementary Fig. I.2A**). Mean standardized uptake value ratio (SUVR) for A β and tau burden were respectively 1.16 $\pm$ 0.08 and 1.32 $\pm$ 0.10 and both showed a statistical trend for a positive association with age (A β : $F_{1,56} = 3.74$, $p = 0.06$; Tau: $F_{1,56} = 3.71$, $p = 0.06$; **Supplementary Fig. I.2B-C**). Furthermore, whole-brain A β burden was strongly associated with whole-brain tau burden ($F_{1,55} = 16.00$, $p = 0.0002$, $R^2_{\beta^*} = 0.23$; **Supplementary Fig. I.2D**).

Cortical excitability dynamics during wakefulness extension

We first investigated wake-dependent changes in cortical excitability during the protocol, using a generalized linear mixed model (GLMM) including random intercept and repeated measurement autoregression [AR(1)]. Cortical excitability during prolonged wakefulness underwent significant changes with time awake after adjusting for age, sex, and education (GLMM, main effect of circadian phase; $F_{4,234.1} = 4.29$, $p = 0.0023$, $R^2_{\beta^*} = 0.07$; **Fig. I.2A**). Post-hoc analyses revealed a global decrease from the beginning to the end of the protocol, with significant differences between the second and last TMS-EEG sessions ($p_{adj} = 0.007$), and between the third and last sessions ($p_{adj} = 0.02$). Visual inspection of individual data indicated, however, an important variability in cortical excitability values and in cortical excitability dynamics. The majority of subjects ($N = 35$, 25 women) displayed an overall decrease in cortical excitability throughout the protocol, whereas ~40% of the sample ($N = 25$, 17 women) exhibited an overall increase in cortical excitability, similar to what was previously reported in young adults (Huber et al., 2013; Ly et al., 2016).

To account for this variability, we summarized cortical excitability dynamics at the individual level using a single value consisting in the regression coefficient of a linear fit across the 5 TMS-EEG measurements (**Fig. I.2B**). Individual residuals indicated that regression quality was good overall and regression coefficients reflected the differences between the first and last sessions of the protocol in most subjects (Supplementary methods). Therefore, the frontal cortical excitability profile (CEP) obtained through the regression fit across the protocol epitomizes cortical excitability dynamics and does not reflect random signal variations. We qualified individuals with positive regression coefficient as young-like CEP, and those with negative regression coefficient as old-like CEP. When considered as two separate groups, young-like and old-like CEP displayed distinct temporal patterns [GLMM, group x circadian phase interaction; $F_{4,234.1} = 13.69$, $p < 0.0001$, $R^2_{\beta^*} = 0.19$; significant between-group post-hoc at circadian phase 30° ($p_{adj} = 0.02$) and 80° ($p = 0.01$); **Supplementary Fig. I.1**], but did not differ in any of the demographic or sleep-wake related variables reported in **Table I.1** ($p > 0.05$). Importantly, frontal CEP was considered as a continuous variable for all statistical analyses reported below and we did not consider young-like and old-like CEP as separate groups.

CEP and sleep slow waves generation

We then confronted the validity of frontal CEP as a measure of sleep-wake regulation by testing its association with slow waves generation during sleep (Skorucak et al., 2018). We found that frontal CEP was significantly related to slow wave energy (SWE), a cumulative measure of slow waves generated during NREM sleep, both in the slower range (0.75-1Hz, $F_{1,55} = 5.35$, $p = 0.02$, $R^2_{\beta^*} = 0.09$, **Fig. I.3A**) and in the higher range (1.25-4Hz, $F_{1,55} = 5.47$, $p = 0.02$, $R^2_{\beta^*} = 0.09$, **Fig. I.3B**), such that young-like frontal CEP was associated with increased, and presumably preserved SWE. We further found that SWE in the lower 0.75-1Hz range was expectedly associated with GM volume ($F_{1,53} = 7.90$, $p = 0.007$, $R^2_{\beta^*} = 0.13$), as well as with whole-brain A β burden ($F_{1,53} = 5.15$, $p = 0.03$, $R^2_{\beta^*} = 0.09$, **Fig. I.3C**), confirming previous reports (Mander et al., 2015; Saletin, van der Helm, & Walker, 2013), but was not linked to whole-brain tau burden ($F_{1,53} = 1.26$, $p = 0.27$). In contrast, CEP was not associated with any of the brain structural integrity measures (GM: $F_{1,53} = 0.18$, $p = 0.67$; A β : $F_{1,53} = 0.16$, $p = 0.69$; Tau: $F_{1,53} = 0.48$, $p = 0.49$). Frontal CEP relates therefore to a gold standard measure of sleep homeostasis known to decline in aging (Carrier et al., 2011; Schmidt et al., 2012) and to be associated with A β burden (Mander et al., 2015), but is not significantly linked to the hallmarks of AD neuropathology.

CEP and cognition

We tested whether frontal CEP and NREM SWE were related to global, memory, attentional, and executive cognitive performance in GLMMs including sex, age, and education as covariates. NREM SWE was not associated with any of the cognitive measures (**Supplementary table I.1**). In contrast, CEP was significantly and positively associated with global cognitive composite score, after accounting for the expected effects of age and education ($F_{1,55} = 6.76$, $p = 0.01$, $R^2_{\beta^*} = 0.11$, **Table I.2, Fig. I.4A**). In other words, individuals displaying preserved frontal cortical excitability dynamics, *i.e.* young-like positive CEP, had better overall cognitive performance, compared to those characterized by old-like negative CEP. Strikingly, we further found a specific and strong positive association between frontal CEP and performance in the executive domain ($F_{1,55} = 8.47$, $p = 0.005$, $R^2_{\beta^*} = 0.13$; **Table I.2, Fig. I.4B**). By contrast, neither memory ($F_{1,55} = 0.41$, $p = 0.52$) nor attention ($F_{1,55} = 2.44$, $p = 0.12$) were associated with CEP (**Table I.2, Fig. I.4C-D**), suggesting that the link between CEP and global cognition mainly arises from the executive domain. Replacing CEP by the difference between the first and last TMS-EGG sessions led to similar statistical outcomes,

ensuring that our findings are not a spurious consequence of the linear regression fit approach and thus reflect the sleep-wake-dependent regulation of the build-up of sleep need on basic brain function (**Supplementary methods; Supplementary table I.2**).

CEP and age-related changes in brain structure

We found no association between the hallmarks of AD neuropathology and global, memory, attentional, and executive cognitive performance (**Table I.3**). Critically, including region-specific brain structural integrity measures in the GLMMs seeking for associations between CEP and cognitive measures did not affect the statistical outcomes (**Table I.3**); if anything, the link between CEP and executive performance became slightly stronger as suggested by semi-partial R^2 values ($R^2_{\beta^*} = 0.13$ vs. $R^2_{\beta^*} = 0.16$). Results were similar if we considered brain structural integrity measures only over the TMS stimulation target area, *i.e.* the superior frontal gyrus (**Supplementary table I.3**). The associations between CEP and cognition may therefore be independent of brain structural integrity measures.

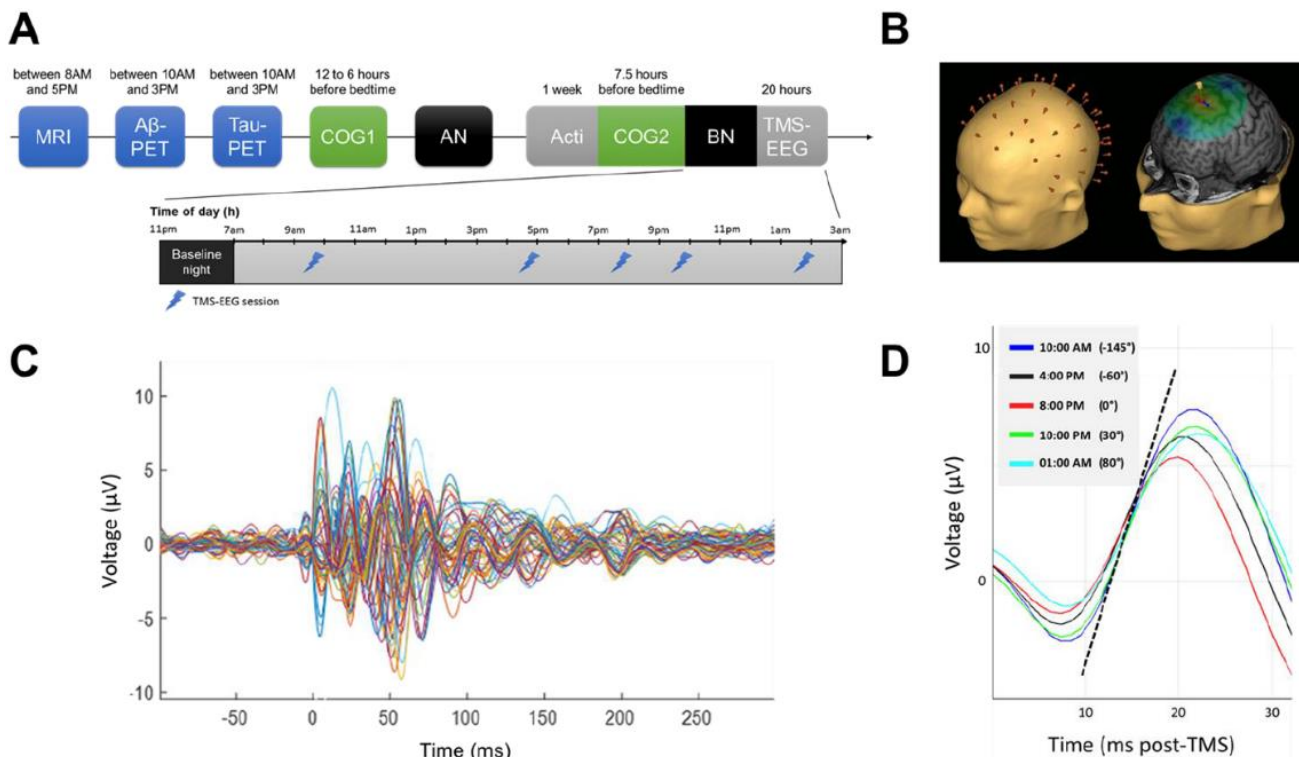


Figure I.1. Study design and cortical excitability assessment. **A)** Overview of the whole experimental protocol and the timing of the different steps for a representative subject with bedtime at 11:00PM and wake time at 07:00AM; AN: adaptation night with polysomnography to screen for sleep apnea; BN: baseline night under EEG recording. **B)** Cortical excitability over the frontal cortex was assessed using neuronavigation-based TMS coupled to EEG. *Left*: reconstructed head with electrodes position; *Right*: representative location of TMS coil and stimulation hotspot with electric field orientation. **C)** Butterfly plot of TMS-evoked EEG response over the 60 electrodes (-100 ms pre-TMS to 300 ms post-TMS; average of ~250 trials). **D)** Representative TMS-evoked EEG potential (0-32 ms post-TMS) in the 5 TMS-EEG sessions with indicative clock time and circadian phase ($15^\circ = 1h$). Cortical excitability was computed as the slope ($\mu V/ms$) of the first component of the TMS-evoked EEG response at the electrode closest to the hotspot (dotted line: example for 10:00AM session).

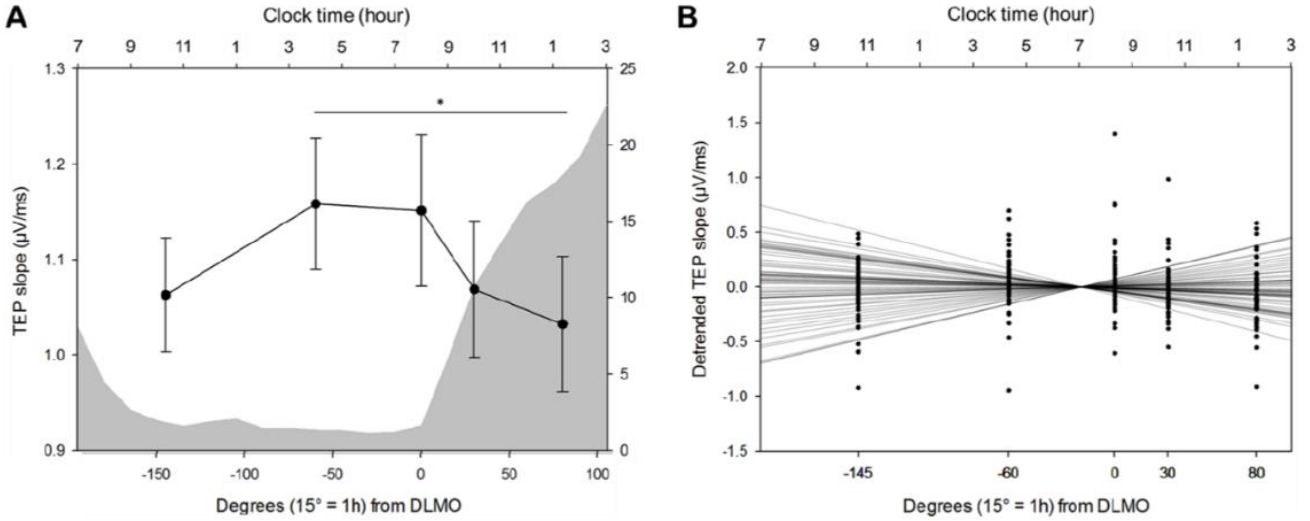


Figure I.2. Cortical excitability dynamics as a marker of sleep-wake regulation processes. A) Average cortical excitability dynamics (mean $\pm$ SEM) during 20h of prolonged wakefulness over the entire sample ($n = 60$). Grey background represents the average melatonin secretion profile (0° indicating dim light melatonin onset, *i.e.* the beginning of the biological night; 15° = 1h). $*p_{\text{adj}} < 0.01$. **B)** Detrended cortical excitability values of all individuals and their respective linear regression lines across the 5 TMS-EEG measurements.

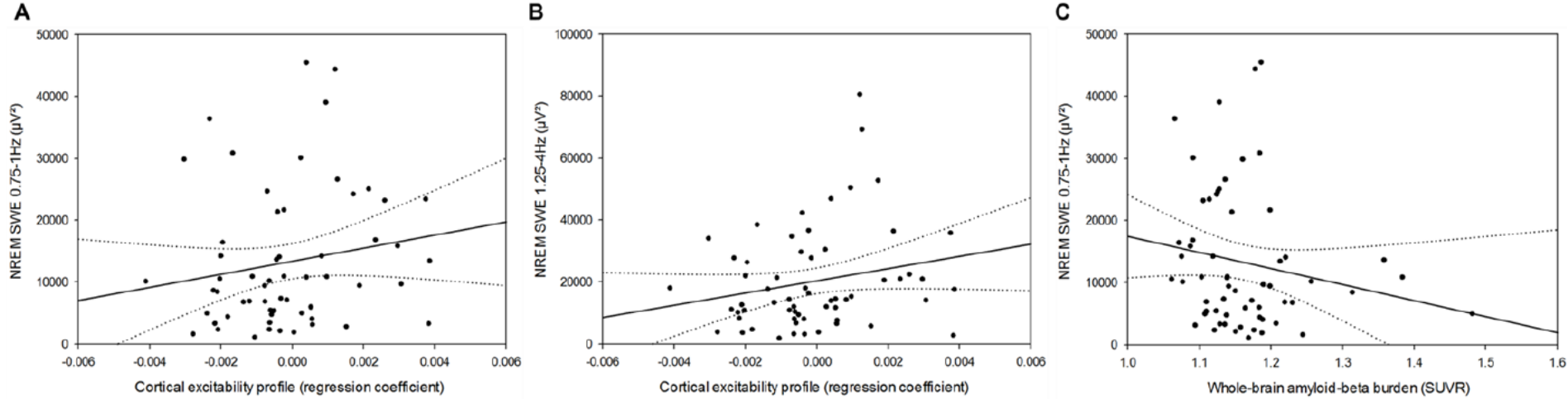


Figure I.3. Cortical excitability, slow wave energy, and brain structural integrity. **A)** Positive association between CEP and cumulated frontal NREM SWE in the lower range (0.75-1Hz) during habitual sleep ($n = 60$; $F_{1,55} = 5.35$, $p = 0.02$, $R^2_{\beta^*} = 0.09$). **B)** Positive association between CEP and cumulated frontal NREM SWE in the higher range (1.25-4Hz) during habitual sleep ($n = 60$; $F_{1,55} = 5.47$, $p = 0.02$, $R^2_{\beta^*} = 0.09$). **C)** Negative association between NREM SWE (0.75-1Hz range) and whole-brain amyloid-beta burden ($n = 60$; $F_{1,53} = 5.15$, $p = 0.03$, $R^2_{\beta^*} = 0.09$). Simple regressions were used only for a visual display and do not substitute the GLMM outputs. Dotted lines represent 95% confidence interval of these simple regressions.

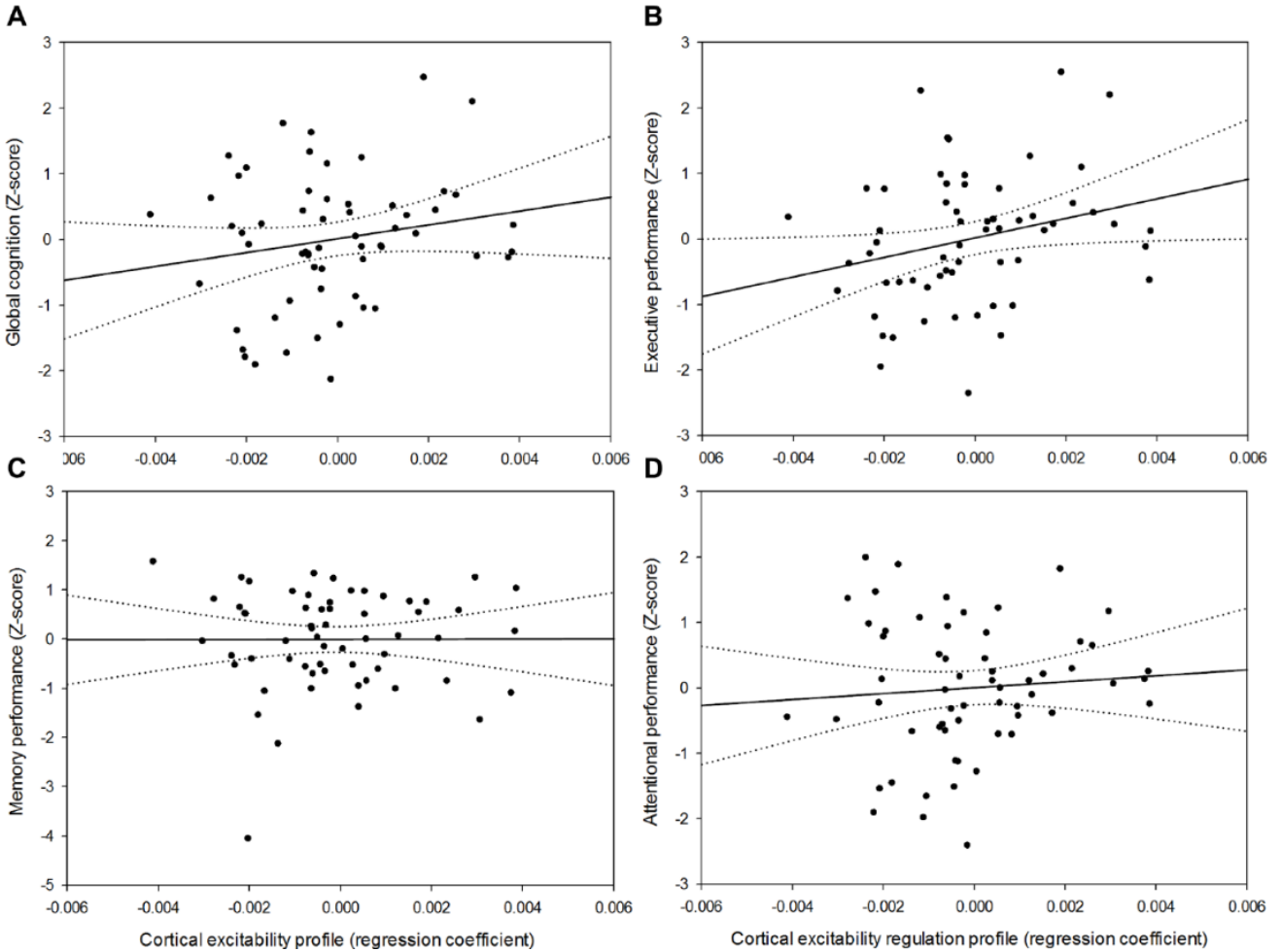


Figure I.4. Relationships between CEP and cognition. **A)** Positive association between CEP and global cognition ($n = 60$; $F_{1,55} = 6.76$, $p = 0.01$, $R^2_{\beta^*} = 0.11$). **B)** Domain-specific positive association between CEP and performance to tasks probing executive functions ($n = 60$; $F_{1,55} = 8.47$, $p = 0.005$, $R^2_{\beta^*} = 0.13$). **C)** No significant association between CEP and memory performance ($n = 60$; $F_{1,55} = 0.39$, $p = 0.54$). **D)** No significant association between CEP and attentional performance ($n = 60$; $F_{1,55} = 2.44$, $p = 0.12$). Simple regressions were used only for a visual display and do not substitute the GLMM outputs. Dotted lines represent 95% confidence interval of these simple regressions.

Table I.1. Sample characteristics (mean $\pm$ SD).

	n = 60
Sex	42w/18m
Age	59.6 $\pm$ 5.5
Education	15.4 $\pm$ 3.2
Right-handed	59
Ethnicity	Caucasian
Dementia Rating Scale	142.1 $\pm$ 2.3
Raven's Progressive Matrices	50.5 $\pm$ 4.9
Mill Hill Vocabulary Scale	26.9 $\pm$ 3.9
Body Mass Index (kg/m ²)	24.6 $\pm$ 2.9
Anxiety	2.6 $\pm$ 2.5
Mood	4.4 $\pm$ 4.7
Caffeine (cups/day)	3.6 $\pm$ 1.9
Alcohol (doses/week)	3.9 $\pm$ 4.0
Treated for hypertension (stable > 6 months)	7
Treated for hypothyroidism (stable > 6 months)	12
Systolic blood pressure (mmHg)	118.74 $\pm$ 11.62
Sleep quality	5.1 $\pm$ 3.0
Daytime sleepiness	6.2 $\pm$ 3.9
Chronotype	53.8 $\pm$ 8.3
Clock time of dim light melatonin onset (hh:min, PM)	08:20 $\pm$ 00:59
In-lab baseline sleep duration (min, EEG)	388.0 $\pm$ 44.3
In-lab baseline sleep efficiency, including N1 stage (% , EEG)	82.6 $\pm$ 9.6
Baseline sleep time (hh:min, PM)	10:47 $\pm$ 00:36
Baseline wake time (hh:min, AM)	06:46 $\pm$ 00:43
Grey matter volume (% of total volume)	41.04 $\pm$ 3.73
[¹⁸ F]Flutemetamol (SUVR)	1.16 $\pm$ 0.08
[¹⁸ F]THK-5351 (SUVR)	1.32 $\pm$ 0.10

Anxiety was measured by the 21-item Beck Anxiety Inventory (Beck, Epstein, Brown, & Steer, 1988); mood by the 21-item Beck Depression Inventory II (Beck, Steer, & G., 1988); caffeine and alcohol consumption by self-reported questionnaires; sleep quality by the Pittsburgh Sleep Quality Index (Buysse et al., 1989); daytime sleepiness by the Epworth Sleepiness Scale (Johns, 1993); chronotype by the Horne-Östberg questionnaire (no participants were extreme chronotypes, *i.e.* scores < 30 or > 70 (Horne & Ostberg, 1976)). Systolic blood pressure was measured in-bed after laying down for > 15 min and 1 to 2h prior to bedtime.

Table I.2. Associations between CEP and cognitive composite scores of global and domain-specific performance adjusted for age, sex, and education. Statistical outputs of Generalized Linear Mixed Models with cognitive scores as dependent measures, accounting for their respective data distribution profiles. $R^2_{\beta^*}$ corresponds to semi-partial R^2 in GLMMs.

	Global performance (Z-score)	Memory (Z-score)	Attentional (Z-score)	Executive (Z-score)
CEP	$F_{1,55} = 6.76$	$F_{1,55} = 0.41$	$F_{1,55} = 2.44$	$F_{1,55} = 8.47$
	$p = 0.01$	$p = 0.52$	$p = 0.12$	$p = 0.005$
	$R^2_{\beta^*} = 0.11$			$R^2_{\beta^*} = 0.13$
Age	$F_{1,55} = 6.97$	$F_{1,55} = 3.76$	$F_{1,55} = 5.79$	$F_{1,55} = 2.68$
	$p = 0.01$	$p = 0.06$	$p = 0.02$	$p = 0.11$
	$R^2_{\beta^*} = 0.11$		$R^2_{\beta^*} = 0.10$	
Sex	$F_{1,55} = 0.01$	$F_{1,55} = 0.02$	$F_{1,55} = 0.05$	$F_{1,55} = 0.11$
	$p = 0.90$	$p = 0.90$	$p = 0.83$	$p = 0.74$
Education	$F_{1,55} = 6.18$	$F_{1,55} = 0.14$	$F_{1,55} = 3.60$	$F_{1,55} = 6.52$
	$p = 0.02$	$p = 0.71$	$p = 0.06$	$p = 0.01$
	$R^2_{\beta^*} = 0.10$			$R^2_{\beta^*} = 0.11$

Table I.3. Associations between CEP and cognitive composite scores of global and domain-specific performance after accounting for global and region-specific brain structural integrity markers.

Statistical outputs of Generalized Linear Mixed Models with cognitive composite scores as dependent measures, accounting for their respective data distribution profiles. When considering global cognitive performance, region-specific A β and tau burden as well as GM density refer to whole-brain values. $R^2_{\beta^*}$ corresponds to semi-partial R^2 in GLMMs.

	Global performance	Memory	Attentional	Executive
	(Z-score)	(Z-score)	(Z-score)	(Z-score)
CEP	$F_{1,52} = 7.49$ $p = 0.009$ $R^2_{\beta^*} = 0.13$	$F_{1,52} = 0.39$ $p = 0.54$	$F_{1,52} = 2.71$ $p = 0.11$	$F_{1,52} = 9.71$ $p = 0.003$ $R^2_{\beta^*} = 0.16$
Age	$F_{1,52} = 7.48$ $p = 0.009$ $R^2_{\beta^*} = 0.13$	$F_{1,52} = 5.51$ $p = 0.02$ $R^2_{\beta^*} = 0.10$	$F_{1,52} = 5.22$ $p = 0.03$ $R^2_{\beta^*} = 0.09$	$F_{1,52} = 3.16$ $p = 0.08$
Sex	$F_{1,52} = 0.04$ $p = 0.84$	$F_{1,52} = 0.29$ $p = 0.59$	$F_{1,52} = 0.01$ $p = 0.95$	$F_{1,52} = 0.75$ $p = 0.39$
Education	$F_{1,52} = 6.47$ $p = 0.01$ $R^2_{\beta^*} = 0.11$	$F_{1,52} = 0.99$ $p = 0.32$	$F_{1,52} = 3.22$ $p = 0.08$	$F_{1,52} = 5.82$ $p = 0.02$ $R^2_{\beta^*} = 0.10$
Region-specific GM volume	$F_{1,52} = 0.11$ $p = 0.75$	$F_{1,52} = 3.54$ $p = 0.07$	$F_{1,52} = 0.04$ $p = 0.85$	$F_{1,52} = 0.10$ $p = 0.75$
Region-specific A β burden	$F_{1,52} = 0.14$ $p = 0.71$	$F_{1,52} = 1.96$ $p = 0.17$	$F_{1,52} = 0.02$ $p = 0.88$	$F_{1,52} = 0.02$ $p = 0.90$
Region-specific Tau burden	$F_{1,52} = 1.17$ $p = 0.28$	$F_{1,52} = 0.66$ $p = 0.42$	$F_{1,52} = 1.07$ $p = 0.31$	$F_{1,52} = 2.51$ $p = 0.12$

Discussion

Healthy aging is accompanied by a disruption of sleep and wakefulness regulation that participates to age-related cognitive decline. Sleep-wake modifications are rooted in part in age-related alterations in brain structure that can lead to AD. Previous investigations found that brain activity during sleep is related to A β burden, and that A β burden affects memory performance through modifications in brain activity during sleep (Mander et al., 2015). This was however reported in elderly individuals (75.1 ± 3.5 years) (Mander et al., 2015), in which A β and tau proteins accumulation as well as neurodegeneration are likely relatively important, while daytime brain activity was not assessed. Here, we confirm that brain activity during sleep is associated with A β burden also in healthy late-middle aged individuals (59.6 ± 5.5 years). Yet, in this relatively large ($N = 60$) younger sample, slow waves generation during sleep is not associated with tau burden nor with cognitive measures including memory, attention, and executive functions. We demonstrate instead that, at around 60 years, wake-dependent variations of basic cortical function is associated with cognitive fitness, independently of A β and tau burden and GM volume.

Previous studies investigating the same stimulation area reported an overall increase in cortical excitability during prolonged wakefulness in young individuals ((Huber et al., 2013), 40h of sleep deprivation, 6 participants aged 25-41 years; (Ly et al., 2016), 29h of sleep deprivation, 24 participants aged 19-30 years), whereas only smaller variations were detected in healthy older individuals ((Gaggioni et al., 2019), 34h of sleep deprivation, 13 young aged 19-27 years and 13 older participants aged 59-69 years). Here, in a larger sample size of older individuals, we showed that a mild wakefulness extension results in significant changes in cortical excitability with time awake. Over the entire sample, cortical excitability globally decreased from the beginning to the end of the protocol, with marked differences between the late-afternoon and the night time sessions. We further show that, amongst healthy older individuals, a large proportion (~40%) of participants displayed an overall wake-dependent increase in frontal cortical excitability which is similar to what was previously reported in young adults (Huber et al., 2013; Ly et al., 2016). We interpret this as a sign of preserved, or young-like, temporal dynamics of cortical excitability, which corresponds to a positive CEP or linear regression coefficient over the entire protocol. Importantly, we find that positive CEP is correlated to increased slow waves generation during habitual sleep, suggesting that individuals with young-like CEP during wakefulness are characterized by a stronger and

potentially preserved sleep homeostasis drive during sleep (Schmidt et al., 2012), compared to individuals with old-like CEP. This strongly suggests that frontal CEP is a measure of the active brain during wakefulness that reflects individuals' preservation of sleep-wake regulation processes.

Critically, we found that higher frontal CEP is associated with better overall cognitive performance, demonstrating a significant relationship between cortical excitability dynamics during wakefulness and cognition, which are both measured in an active and awake brain. In-depth cognitive phenotyping showed that this relationship was mainly driven by the performance in the executive domain. Executive functions refer to high-order cognitive processes (flexibility, inhibition, updating, etc.) needed for behavioral adjustment according to ongoing goals when facing new or complex situations (Jurado & Rosselli, 2007). Variations in executive performance assessed during sleep deprivation were previously found to be associated with cortical excitability dynamics in older and younger individuals (Gaggioni et al., 2019). Here, we further show that cognitive ability, as an individual trait measured outside a sleep deprivation protocol, is significantly associated with sleep-wake regulation of frontal cortical excitability. Executive functions influence other cognitive domains and are often seen as central in age-related cognitive decline to remain adapted to the environment and sustain day-to-day functioning in complete autonomy (Niendam et al., 2012). In addition, executive functions are considered to depend mainly on the frontal cortex processes, and their underlying cortical networks undergo important changes in healthy aging (Podell et al., 2012). These results therefore suggest that preserved frontal CEP may constitute a marker of cognitive fitness in aging, and particularly so in the executive domain.

Despite the relative youth of our sample (~60 years), the absence of association between cognitive measures and brain integrity markers of GM volume and protein burdens may appear surprising. Longitudinal studies have shown, however, that the association between some aspects of cognition and brain structure was especially apparent in participants aged 65 years and over (Gorbach et al., 2016). Subtle differences in cognitive performance in relation to proteins accumulation or GM reduction may therefore not appear in our sample. This may also underlie the absence of significant association between sleep slow waves generation and tau burden that has been recently reported in an older sample of healthy individuals (73.8 ± 5.3 years) (Lucey et al., 2019). In addition, our sample is biased towards individuals with higher education (mean 15.5 ± 3.2 years) and with cognitive and brain reserves that may compensate for early alteration of brain structural integrity (Reuter-Lorenz

& Park, 2014; Y. Stern, 2012). Alternatively, the composite scores for each cognitive domain may not be sensitive enough to be related to these early brain alterations. Nonetheless, our findings indicate that sleep-wake regulation of brain activity during wakefulness, as measured by the dynamics of cortical excitability during a mild wakefulness extension, is either more sensitive than brain structural integrity markers to isolate associations with cognition in aging, or sensitive to aspects of cognition that undergo influences distinct from protein accumulations and neurodegeneration. Another explanation to our results might involve the soluble forms of A β and tau, as oligomers of both proteins were shown to alter neuronal function (Kopeikina et al., 2012; Masters & Selkoe, 2012). Currently these oligomers cannot be reliably measured *in vivo* and could not be accounted for in this experiment.

Furthermore, age-related molecular changes potentially underlying sleep need have been reported (Meyer et al., 2007). These may influence the local or global genetic and molecular machineries underlying circadian rhythmicity and sleep homeostasis, and in turn affect cortical excitability. Aging modulates the impact of the basal forebrain, subcortical and brainstem ascending activating system on global brain activity (Scammell et al., 2017). *In silico* modeling of wake-dependent cortical excitability changes in young adults suggests that the fluctuations in the balance between excitation and inhibition within cortical networks may affect the observed variations during prolonged wakefulness (Chellappa et al., 2016). However, this remains unexplored in healthy older individuals. It might also be the case that changes in cortical function stems from AD-related alteration of subcortical structures. In addition to the locus coeruleus (Braak, Thal, Ghebremedhin, & Del Tredici, 2011), neurodegeneration of the suprachiasmatic nuclei, site of the master circadian clock, has been reported in AD (J. L. Wang et al., 2015) while network uncoupling in the suprachiasmatic nuclei is found in normal aging (Farajnia et al., 2014). Future investigations should also examine whether cortical excitability dynamics, probed specifically over the frontal regions, are related to cognitive changes in aging when compared to cortical excitability dynamics probed over other parts of the brain and to other aspects of cognitive brain functions. Furthermore, the predictive value of frontal CEP assessment for subsequent cognitive decline and risk of developing dementia remains to be investigated in a longitudinal protocol.

This study presents several strengths. The use of TMS-EEG allows for a direct measure of cortical responsiveness while bypassing sensory systems and it mimics active brain processing without confounding biases. The prolonged wakefulness protocol is performed under strictly controlled constant routine conditions to control for multiple factors

that could affect wakefulness and sleep parameters, such as light exposure or physical activity (Duffy & Dijk, 2002). In addition, we performed a comprehensive multi-modal assessment of brain structure, including two PET scans and MRI for the hallmarks of AD pathophysiology, as well as an extensive neuropsychological investigation. Furthermore, sleep-wake history was controlled prior to wake extension and exclusion criteria ensured most risk factor favoring cognitive decline were not present in the sample (e.g. diabetes, smoking, alcohol abuse, depression, etc.) (Norton et al., 2014). Finally, the relative young age of the participants reduces the accumulation of minor health issues associated with advanced age (e.g. diabetes, overweight, etc.), which can inherently affect findings in samples of elderly individuals. This research also has several limitations, however. Its cross sectional nature does not allow us to comment on the future cognitive trajectory of participants. The reported effect sizes show that CEP does explain but a small part of variance, suggesting that its link with cognitive fitness is modest. This was expected given the numerous factors that affect cognitive trajectories (Norton et al., 2014) and the relatively young age and good overall health status of our sample. It is, in fact, quite remarkable that we were nonetheless able to isolate a link between wake-dependent cortical excitability dynamics and cognition in such sample, suggesting that it may be a very important link to successful cognitive aging. While the contribution of the homeostatic process is most obvious in our data, a longer protocol covering the whole circadian cycle would help disentangle the respective modulation of frontal CEP by the circadian system (Duffy & Dijk, 2002). In addition, [¹⁸F]THK-5351 presents some unspecific binding, particularly around the fornix and basal ganglia (Villemagne et al., 2018). We took this into account by excluding these portions of the brain from all tau burden measures. The observed links between [¹⁸F]THK-5351 uptake and both age and Aβ burden strongly support that our measure of tau burden, although potentially imperfect, was meaningful. Finally, we did not consider other age-related changes of brain integrity, such as cerebrovascular pathology, which are extremely common (up to 50%) as a mixed pathology in individuals with Alzheimer's dementia (Alzheimer's Association, 2016), and Lewy bodies pathology, which shares some genetic risk with Alzheimer's disease (Guerreiro et al., 2016).

Aging is the ultimate challenge that the brain has to face in order to maintain optimal cognition across the lifespan. The bidirectional detrimental interaction between disturbed sleep-wake regulation and AD pathogenesis suggest that sleep-wake interventions could be promising means to reduce the risk of dementia (Van Egroo, Narbutas, Chylinski, Villar González, Maquet, Salmon, Collette, et al., 2019). Here, we provide compelling evidence that

sleep-wake regulation influences cognition in healthy older individuals, particularly in the executive domain, and beyond the changes in brain structural integrity that can ultimately lead to dementia. Since both sleep homeostasis and circadian rhythmicity show significant alteration as early as age 40 (Carrier et al., 2011; Schmidt et al., 2012), our results further reinforces the idea that sleep and wakefulness could be acted upon to improve individual cognitive health trajectory early in the lifespan. Our findings could have therefore implications for the understanding of brain mechanisms underlying the maintenance of cognitive health in normal and pathological aging, and for potential early intervention targets.

Methods

Study design and participants

Between June 15, 2016, and July 28, 2018, healthy older individuals aged 50-70 years were enrolled for this multi-modal cross-sectional study after giving their written informed consent, and received a financial compensation. This research was approved by the Ethics Committee of the Faculty of Medicine at the University of Liège, Belgium. Exclusion criteria were: clinical symptoms of cognitive impairment (Dementia rating scale < 130; Mini mental state examination < 27); Body Mass Index ≤ 18 and ≥ 29 ; recent psychiatric history or severe brain trauma; addiction, chronic medication affecting the central nervous system; hypertension; smoking, excessive alcohol (> 14 units/week) or caffeine (> 5 cups/day) consumption; shift work in the past 6 months; transmeridian travel in the past two months; anxiety, as measured by the 21-item self-rated Beck Anxiety Inventory (score ≥ 10); depression, as assessed by the 21-item self-rated Beck Depression Inventory (score ≥ 14). Participants with stable treatment (for > 6 months) for hypertension and/or hypothyroidism were included in the study. Participants with sleep apnea (apnea-hypopnea index ≥ 15 /hour) were excluded based on an in-lab adaptation and screening night of polysomnography. One participant was excluded from the sample for all analyses because of outlier values on both PET assessments (> 6 standard deviations from the mean). Demographic characteristics of the final study sample are described in **Table I.1**.

Magnetic resonance imaging

High-resolution structural MRI was performed on a 3-Tesla MR scanner (MAGNETOM Prisma, Siemens). For each participant, multi-parameter mapping volumes (*i.e.* T1-weighted, proton density-weighted, magnetization transfer (MT)-weighted) were acquired. We estimated individuals' total intracranial volume and whole-brain GM volume based on the MT-weighted image, using the SPM12 toolbox (<https://www.fil.ion.ucl.ac.uk/spm/>). For regional quantification of GM, volumes of interest were first determined using the Automated Anatomical Labeling atlas (AAL2) (Tzourio-Mazoyer et al., 2002). MT-weighted images were spatially normalized into a study-specific template with the Diffeomorphic Anatomical Registration Through Exponentiated Algebra (DARTEL) toolbox (Ashburner, 2007). Volumes of interest were then applied on segmented

normalized MT-weighted images and combined to extract GM volume in brain regions underlying each cognitive domain (**Supplementary table I.4**).

PET imaging

A β -PET imaging was performed with [^{18}F]Flutemetamol, and tau-PET imaging was done with [^{18}F]THK-5351. For both radiotracers, all participants received a single dose of their respective radioligands in an antecubital vein (target dose 185 MBq). A β -PET image acquisitions started 85 minutes after injection, and 4 frames of 5 minutes were obtained, followed by a 10-minute transmission scan. For tau-PET, transmission scan was acquired first and dynamic image acquisitions started immediately after injection, consisting in 32 frames (with increasing time duration). All PET images were reconstructed using filtered back-projection algorithm including corrections for measured attenuation, dead time, random events, and scatter using standard software (ECAT 7.1, Siemens/CTI, Knoxville, TN). Motion correction was performed using automated realignment of frames without reslicing. A PET sum image was created using all frames for A β -PET, and using the 4 frames corresponding to the time window between 40 and 60 min for tau-PET. PET sum images were reoriented manually according to MT-weighted structural MRI volume and coregistered to structural MRI using the MT-weighted volume. PET sum images were further corrected for partial volume effect (PETPVC toolbox, iterative Yang method (Thomas et al., 2016)) and spatially normalized using the MRI study-specific template. Standardized uptake value ratio (SUVR) was calculated using the cerebellum GM as the reference region. The volumes of interest used for GM analysis were applied to normalized PET sum images to estimate regional SUVR of each radiotracer in cognitive domain-specific regions. [^{18}F]THK-5351 radiotracer shows some unspecificity for tau, particularly over the basal ganglia, which was taken into account by excluding basal ganglia from all computations of PET SUVR values for tau-PET.

Cognitive assessment

Upon arrival for the wake-extension protocol and prior to being placed in dim-light (~7.5 hours before habitual bedtime, corresponding to ~3:30PM for a representative subject with bedtime at 11:00PM), participants were administered the first part (~1h) of the extensive neuropsychological assessment including: 1) Mnemonic Similarity Task ; 2) Category Verbal Fluency (letter and animals); 3) Digit Symbol Substitution Test; 4) Visual N-Back (1-, 2-*, and 3-back variants); and 5) Choice Reaction Time. On another day while well-rested and during the day (from 12 to 6 hours before habitual bedtime, *i.e.* between 11:00AM and

5:00PM for the same representative subject), the second part of the neuropsychological assessment was administered. This ~1.5h session included: 1) Direct and Inverse Digit Span; 2) Free and Cued Selective Reminding Test; 3) Stroop Test; 4) Trail Making Test (part A and B); and 5) D2 Attention Test. The memory function composite score included Free and Cued Selective Reminding Test (sum of all free recalls) and Mnemonic Similarity Task (recognition memory score). The executive function composite score comprised verbal fluency tests (2-min score for letter and animal variants), the digit span (inverse order), Trail Making Test (part B), N-Back (3-back variant), and Stroop (number of errors for interfering items). The attentional function composite score included Digit Symbol Substitution Test (2-min score), Trail Making Test (part A), N-Back (1-back variant), D2 (Gz - F score), and Choice Reaction Time (reaction time to dissimilar items). We computed a composite score for each cognitive domain based on the sum of Z-scores on domain-related tasks, with higher scores reflecting better performance. Composite score for global cognitive performance score consisted of the standardized sum of the domain-specific composite scores.

Sleep assessment and spectral power analysis

As part of the screening process, participants first performed an in-lab adaptation and screening night to minimize the disrupting effect caused by sleeping in a novel environment (Tamaki, Bang, Watanabe, & Sasaki, 2016), which might have otherwise affected the sleep parameters subsequently assessed during the baseline night. Then, for 7 days prior to the wake-extension protocol, participants followed a regular sleep-wake schedule (± 30 min), in agreement with their preferred bed and wake-up times. Compliance was verified using sleep diaries and wrist actigraphy (Actiwatch[®], Cambridge Neurotechnology, UK). Aside from the fixed sleep-wake schedule, participants were also instructed to abstain from intense physical exercise for the last 3 days of fixed-schedule circadian entrainment (*i.e.* right before the wake-extension protocol; exercise/fitness levels were not controlled for more specifically). The day before the wake-extension protocol, participants arrived to the laboratory 8 hours before their habitual bedtime and were kept in dim light (< 5 lux) for 6.5 hours preceding bedtime. Their habitual sleep was then recorded in complete darkness under EEG (baseline night, **Fig. I.1A**). Baseline night data were acquired using N7000 amplifiers (EMBLA, Natus Medical Incorporated, Planegg, Germany). The electrode montage consisted of 11 EEG channels (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1, O2), 2 bipolar electrooculograms, and 2 bipolar electromyograms. Scoring of baseline night in 30-s epochs was performed automatically using a validated algorithm (ASEEGA, PHYSIP, Paris, France) (Berthomier et al., 2007). An

automatic artefact detection algorithm with adapting thresholds (Coppieters 't 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, both for the lower range (0.75-1Hz) and higher range (1.25-4Hz), during all the NREM 2 and NREM 3 epochs of the given period, after adjusting for the number of NREM 2 and 3 epochs to account for artefacted data. As the frontal regions are most sensitive to sleep-wake history (Schmidt et al., 2012), SWE was considered over the frontal electrodes (F3, Fz, F4).

Wake-extension protocol

The wake-extension protocol followed the baseline night and consisted of 20h of continuous wakefulness under strictly controlled constant routine conditions, *i.e.* in-bed semi-recumbent position (except for scheduled bathroom visits), dim light < 5 lux, temperature ~19°C, regular isocaloric food intake, no time-of-day information, and sound-proofed rooms. The protocol schedule was adapted to individual sleep-wake time, and lasted up to the theoretical mid-sleep time (e.g. ca. 07:00 AM-03:00 AM) to create a moderate wakefulness extension challenge. Hourly saliva samples were collected for subsequent melatonin assays, allowing *a posteriori* data realignment and interpolation based on individual endogenous circadian timing. Importantly, participants were not informed about the schedule of the different events included in the wake-extension protocol (e.g. number and timing of scheduled TMS-EEG sessions, saliva samples collections, food intakes, etc.), nor about the exact duration of the protocol in order to avoid motivational/expectancy biases which might interfere with the wake-dependent effects on our measurements (Hull, Wright, & Czeisler, 2003). They were told during recruitment that the wake-extension protocol lasted for around 20h, until the middle of the night. Cortical excitability over the frontal cortex was measured 5 times throughout the protocol, using TMS-EEG, with increased frequency around the circadian wake-maintenance zone, as it represents a critical period around which the interplay between sleep homeostasis and the circadian system show important changes. Participants were instructed that that the protocol included a few TMS-EEG session but were not informed about the exact number.

TMS-EEG assessment

One TMS-EEG session was performed prior to the wake-extension protocol to determine optimal stimulation parameters (*i.e.* location, orientation, and intensity) that allowed for EEG recordings free of muscular and magnetic artefacts. As in previous experiments (Gaggioni et al., 2019; Huber et al., 2013; Ly et al., 2016), the target location was in the superior frontal gyrus. For all TMS-EEG recordings, pulses were generated by a Focal Bipulse 8-Coil (Nexstim, Helsinki, Finland). Interstimulus intervals were randomized between 1900 and 2200 ms. TMS-evoked responses were recorded with a 60-channel TMS-compatible EEG amplifier (Eximia, Helsinki, Finland), equipped with a proprietary sample-and-hold circuit which provides TMS artefact free data from 5 ms post stimulation. Electrooculogram was recorded with two additional bipolar electrodes. EEG signal was band-pass filtered between 0.1 and 500 Hz and sampled at 1450 Hz. Before each recording session, electrodes impedance was set below 5 k Ω . Each TMS-EEG session included ~250 trials (mean = 252 ± 15). Auditory EEG potentials evoked by the TMS clicks and bone conductance were minimized by diffusing a continuous white noise through earphones and applying a thin foam layer between the EEG cap and the TMS coil. A sham session, consisting in 30 TMS pulses delivered parallel to the scalp with noise masking, was administered to verify the absence of auditory EEG potentials. TMS-EEG data were preprocessed as previously described (Gaggioni et al., 2019) in SPM12 implemented in MATLAB2013a (The Mathworks Inc., Natick, MA). In brief, TMS-EEG data underwent semi-automatic artefacts rejection, low-pass filtering at 80 Hz, downsampling to 1000 Hz, high-pass filtering at 1 Hz, splitting into epochs spanning -101 and 300 ms around TMS pulses, baseline correcting (from -101 to -1 ms pre-TMS), and robust averaging. Cortical excitability was computed as the slope at the inflexion point of the first component of the TMS-evoked EEG potential on the electrode closest to the stimulation hotspot. For each participant, CEP value was defined as the regression coefficient of a linear fit (first order polyfit MATLAB function) across the 5 TMS-EEG measurements at the individual level.

Melatonin assessment

Salivary melatonin was measured by radioimmunoassay. The detection limit of the assay for melatonin was 0.8 ± 0.2 pg/l using 500 μ l volumes. Dim light melatonin onset times were computed for each participant using the hockey-stick method, with ascending level set to 2.3 pg/ml (Hockey-Stick software v1.5) (Danilenko, Verevkin, Antyufeyev, Wirz-Justice, &

Cajochen, 2014). The circadian phase of all TMS-EEG data points was estimated relative to individual dim light melatonin onset time (*i.e.* phase 0°; 15° = 1h). Based on this, cortical excitability measures were resampled following linear interpolation at the theoretical phases of the TMS-EEG sessions of the protocol (-145°, -60°, 0°, 30°, 80°).

Statistics

Statistical analyses were performed using Generalized Linear Mixed Models (GLMMs) in SAS 9.4 (SAS Institute, Cary, NC). Dependent variables distribution was first determined using `allfitdist` function in MATLAB and GLMMs were adjusted accordingly. All statistical models were adjusted for age, sex and education. Statistical significance was set at $p < 0.05$. Simple regressions were used for visual display only and not as a substitute of the full GLMM statistics. Degrees of freedom were estimated using Kenward-Roger's correction. *P*-values in post-hoc contrasts (difference of least square means) were adjusted for multiple testing with Tukey's procedure. Semi-partial R^2 ($R^2_{\beta^*}$) values were computed to estimate effect size of significant fixed effects in all GLMMs (Jaeger, Edwards, Das, & Sen, 2017a).

Chapter II

Brainstem tau protein burden is associated with cortical hyper-excitability in healthy aging.

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Abstract

Alzheimer's disease (AD) neuropathology spreads over decades prior to cognitive symptom onset. Its first signs consist in misfolded tau protein accumulation in the brainstem, particularly in the locus coeruleus (LC). Neuronal hyper-excitability also occurs early in the pathogenesis of AD and contributes to neuronal network alteration. Here, we tested whether early cortical hyper-excitability may be related to tau aggregates in the brainstem. We probed cortical excitability over the frontal cortex using transcranial magnetic stimulation combined with electroencephalography, and we assessed whole-brain tau protein burden with [¹⁸F]THK5351 positron emission tomography in a sample of 65 late middle-aged healthy individuals (50-70y). We found that higher tau burden in a brainstem monoaminergic grey matter compartment, including notably the LC, was associated with increased cortical excitability. By contrast, tau deposits in the hippocampus and entorhinal cortex, although correlating with brainstem tau signal, was not associated with cortical excitability. This suggests a specific association between cortical excitability and brainstem tau aggregates. Diffusion-weighted imaging-based tractography further revealed that frontal cortical excitability was linked to the integrity of the tracts linking the frontal cortex and a dorsal pontine tegmentum area encompassing the LC. These findings reveal potential brain substrates for increased cortical excitability in preclinical and early AD, and they constitute the first human functional correlates of *in vivo* early tau protein accumulation in the brainstem.

Introduction

Alzheimer's disease (AD) is characterized by neuropathological processes that spread over decades prior to cognitive symptoms onset (Jack et al., 2018). Tau neurofibrillary tangles (NFT) and amyloid-beta ($A\beta$) senile plaques play a central role in this process (Braak & Tredici, 2015). *Post-mortem* studies showed that accumulation of misfolded hyperphosphorylated tau protein that eventually assembles into NFT appears prior to any detectable $A\beta$ lesion (Braak, Thal, Ghebremedhin, & Tredici, 2011). By the age of 30, a vast majority of the population presents hyperphosphorylated tau aggregates in the locus coeruleus (LC), a small noradrenergic brainstem nucleus (Mather & Harley, 2016), sometimes concomitantly to other brainstem nuclei such as the dorsal raphe (Braak & Del Tredici, 2011; Braak, Thal, Ghebremedhin, & Tredici, 2011; Ehrenberg et al., 2017). Tau aggregates then spread to the entorhinal and hippocampal regions, before following stereotypical outward progression pattern in the cortex (Braak, Thal, Ghebremedhin, & Tredici, 2011).

Neuronal hyper-excitability occurs early in the pathogenesis of AD and contributes to neuronal network alterations (D'Amelio & Rossini, 2012; Tracy & Gan, 2018). Motor cortex hyper-excitability has been reported in prodromal AD (Mild Cognitive Impairment - MCI) (Ferreri et al., 2011; Olazarán, Prieto, Cruz, & Esteban, 2010), while acetylcholinesterase inhibitor therapy has been suggested to restore normal cortical excitability in AD patients (Trebbastoni et al., 2016). In advanced AD, *i.e.* when it is likely that tau and $A\beta$ protein burdens are high and that important neurodegeneration takes place, cortical excitability decreases markedly (Casarotto et al., 2011). The mechanisms underlying increased cortical excitability in AD preclinical phase are not established but they may involve tau protein.

Under non-pathological conditions, tau is a micro-tubule associated protein that stabilizes the axonal cytoskeleton and plays an important role in synaptic function (Mietelska-Porowska et al., 2014). The release of tau in the extracellular space, which seems to underlie the propagation of tau pathology across anatomically interconnected networks (Yamada, 2017), is regulated by neuronal activity (Yamada et al., 2014). Elevated interstitial and cerebrospinal (CSF) fluid tau levels have been observed during periods of extended neuronal activity such as sleep deprivation (Holth et al., 2019). In AD animal models, tau pretangles and their surrounding soluble hyperphosphorylated form, as well as NFTs, are known to have a deleterious impact on neuronal function (Tracy & Gan, 2018). Several studies reported a

direct relationship between tau protein accumulation and neurophysiological alteration of neuronal network excitability in rodents and drosophila models (Hall et al., 2015; Holth et al., 2013; Schultz et al., 2018). Furthermore, tau pathology appears to propagate from early aggregation sites to remote brain locations through direct axonal connections (De Calignon et al., 2012).

Tau pretangles and soluble hyperphosphorylated forms remain undetectable *in vivo* in humans, so their impact on brain function is unknown. In contrast, tau NFT detection has been made possible with the recent advent of novel Positron Emission Tomography (PET) radioligands (Murugan et al., 2019). Tau pretangles can be observed in *post mortem* tissues in youth, but NFT are present in about half of the population only starting at the sixth decade of life (Braak, Thal, Ghebremedhin, & Tredici, 2011). Here, our main goal was to assess whether cortical excitability was associated with the presence of tau NFT in the brainstem, *i.e.* in the first site of misfolded tau accumulation, among late middle-age cognitively normal individuals (50 to 70 y). We directly measured cortical excitability using electroencephalogram (EEG) recordings of brain responses to transcranial magnetic stimulation (TMS), which mimics the active brain processing external stimulation. We assessed whole brain tau NFT burden using PET imaging and, based on quantitative magnetic resonance imaging (MRI) data, we then extracted tau radiotracer uptake values in a compartment of the brainstem including monoaminergic neurons. Using diffusion-weighted magnetic resonance imaging (DWI)-based tractography, we further explored whether a putative association between cortical excitability and tau could be related to the integrity of the white matter tracts connecting the brainstem to the cortex.

Materials and Methods

Experimental design

Healthy older individuals aged 50-70 years were enrolled in a multi-modal cross-sectional study investigating the relationships between AD neuropathology, wake-dependent cortical excitability dynamics, and cognitive aging. In that context, we measured cortical excitability over the frontal cortex using TMS-EEG (**Fig. II.1A-B**), and we assessed whole-brain tau protein burden with [¹⁸F]THK5351 PET imaging. All participants also underwent quantitative multiparametric and diffusion-weighted MRI acquisitions for subsequent brainstem segmentation and tractography analyses (Weiskopf et al., 2015).

Participants

66 healthy older individuals (mean age = 59.60 ± 5.36 years; 42 women) took part in this research, which was approved by the Ethics Committee of the Faculty of Medicine at the University of Liège, Belgium. Participants gave their written informed consent and received a financial compensation. Exclusion criteria for the study were: clinical symptoms of cognitive impairment (Dementia rating scale < 130 (S. Mattis, 1976); Mini mental state examination < 27); Body Mass Index (BMI) ≤ 18 and ≥ 29 ; recent psychiatric history or severe brain trauma; addiction, chronic medication affecting the central nervous system; smoking, excessive alcohol (> 14 units/week) or caffeine (> 5 cups/day) consumption; shift work in the past 6 months; transmeridian travel in the past two months; high levels of anxiety, as measured by the 21-item self-rated Beck Anxiety Inventory (BAI ≥ 17) (Beck, Epstein, et al., 1988); high levels of depression, as assessed by the 21-item self-rated Beck Depression Inventory (BDI ≥ 17) (Beck, Steer, et al., 1988). Participants with sleep apnea (apnea-hypopnea index ≥ 15 /hour) were excluded based on an in-lab screening night of standard polysomnography. One participant was excluded from the study sample because of outlier values on whole-brain ^{18}F -THK5351 PET assessment (> 4 standard deviations from the mean). Demographic characteristics of the final study sample are described in **Table II.1**.

Experimental protocol

For 7 days prior to TMS-EEG protocol, participants followed a regular sleep-wake schedule (± 30 min), in agreement with their preferred bed and wake-up times. Compliance was verified using sleep diaries and wrist actigraphy (Actiwatch®, Cambridge Neurotechnology, UK). Aside from the fixed sleep-wake schedule, participants were also instructed to abstain from unusual physical exercise as well as caffeine and alcohol consumption for the last 3 days of fixed sleep-wake schedule. The day before the experiment, participants arrived to the laboratory 8 hours before their habitual bedtime and were kept in dim light (< 5 lux) for 6.5 hours preceding bedtime.

TMS-EEG assessments were performed the next day in the context of a 20h protocol of wakefulness extension in strictly controlled constant routine conditions, *i.e.* in-bed semi-recumbent position (except for scheduled bathroom opportunities), dim light < 5 lux, temperature $\sim 19^\circ\text{C}$, regular isocaloric food intake, no time-of-day information, and sound-proofed rooms. The protocol schedule was adapted to individual sleep-wake time, and lasted up to the theoretical mid-sleep time (e.g. ca. 07:00 AM-03:00 AM). Cortical excitability over

the frontal cortex was measured 5 times throughout the protocol. Here, we only considered the first assessment of cortical excitability recorded 3 hours after wake-up time (± 0.21 hours). Other considerations related to repeated TMS-EEG sessions are reported elsewhere (Gaggioni et al., 2019).

TMS-EEG signal acquisition and processing

Optimal stimulation parameters (*i.e.* location, orientation, and intensity) were determined during a separate TMS-EEG session carried out prior to the experimental protocol, and allowed for EEG recordings free of muscular and magnetic artefacts. As in previous experiments (Gaggioni et al., 2019; Huber et al., 2013; Ly et al., 2016), the target location was in the superior frontal gyrus (**Fig. II.1A**). For all TMS-EEG recordings, pulses were generated by a Focal Bipulse 8-Coil (Nexstim, Helsinki, Finland). Interstimulus intervals were randomized between 1900 and 2200 ms. TMS-evoked responses were recorded with a 60-channel TMS-compatible EEG amplifier (Eximia, Helsinki, Finland), equipped with a proprietary sample-and-hold circuit which provides TMS artefact-free data from 5 ms post stimulation (Virtanen, Ruohonen, Näätänen, & Ilmoniemi, 1999). Electrooculogram (EOG) was recorded with two additional bipolar electrodes. EEG signal was band-pass filtered between 0.1 and 500 Hz and sampled at 1450 Hz. Before each recording session, electrodes impedance was set below 5 k Ω . Each TMS-EEG session included ~250 trials. Auditory EEG potentials evoked by the TMS clicks and bone conductance were minimized by diffusing a continuous white noise through earphones and applying a thin foam layer between the EEG cap and the TMS coil.

TMS-EEG data were pre-processed as previously described (Gaggioni et al., 2019; Ly et al., 2016) in Statistical Parametric Mapping 12 (SPM12, <http://www.fil.ion.ucl.ac.uk/spm/>) implemented in MATLAB2013a (The Mathworks Inc., Natick, MA, USA). In brief, TMS-EEG data underwent semi-automatic artefacts rejection, low-pass filtering at 80 Hz, downsampling to 1000 Hz, high-pass filtering at 1 Hz, splitting into epochs spanning -101 and 300 ms around TMS pulses, baseline correcting (from -101 to -1 ms pre-TMS), and robust averaging. Cortical excitability was defined as the slope at the inflexion point of the first component (0-35 ms) of the TMS-evoked EEG potential (TEP) measured on the artefact-free electrode closest to the target stimulation location. Median position of the closest artefact-free recording electrode was [X: -34; Y: -3.7; Z: 85.9, mm, Montreal Neurological Institute (MNI) space]. For the present paper and to avoid any wake-

dependent modulation of cortical excitability (Gaggioni et al., 2019), we only used cortical excitability values assessed during the first session.

Quantitative multi-parametric acquisition and processing

Structural and quantitative maps of T1, T2*, proton density (PD) and magnetization transfer (MT) at 1 mm resolution were calculated from a multi-parameter protocol based on a 3D multi-echo fast low angle shot (FLASH) sequence (Weiskopf & Helms, 2008). Three co-localised 3D multi-echo FLASH data sets were acquired with predominantly proton density weighting (PDw: TR/FA=23.7 ms/6°), T1 weighting (T1w: TR/FA=18.7 ms/20°), and MT weighting (MTw: TR/FA=23.7 ms/6°; excitation preceded by an off-resonance Gaussian MT pulse of 5 ms duration, 220° nominal flip angle, 2 kHz frequency offset) in a total acquisition time of approx. 19 min, with a voxel size of 1 mm³ isotropic. Two calibration sequences were acquired to correct for inhomogeneities in the radio frequency (RF) transmit field.

Quantitative multi-parametric maps (MT, PD, R1, R2*) were generated with the hMRI toolbox (Tabelow et al., 2019) (<http://hmri.info>) implemented in MATLAB2013a. First, MT and PD maps were segmented into grey, white, and CSF tissue class maps using Unified Segmentation (US) within SPM12 (Ashburner & Friston, 2005). Whole-brain segmentation outputs were diffeomorphically registered to a study-specific template, compatible with the MNI space, created using Shoot toolbox in SPM12 (Ashburner & Friston, 2011) in order to generate deformation fields that were used to warp MT and PD maps into the Shoot common-average space. Brainstem segmentation was then performed using US with brainstem sub-regions tissue probability maps that were generated according to previously described methods based on a modified multivariate mixture of Gaussians (C. Lambert, Lutti, Helms, Frackowiak, & Ashburner, 2013). Out of the four brainstem tissue classes produced, we considered only tissue class 1 which encompassed monoaminergic grey matter including LC and raphe nuclei. Tissue class 1 was further masked into a dorsal meso-pontine tegmentum region (DTR) to increase its focus around the LC, with rostro-caudal boundaries placed between the inferior colliculus and the superior olivary nucleus, antero-posterior boundaries between the medial longitudinal fasciculus and the anterior periaqueductal grey matter, and lateral boundaries between the lateral lemnisci (**Fig. II.1C**), using the ‘Snake’ function in ITK-Snap 3.8 (Yushkevich et al., 2006). Brainstem monoaminergic grey matter (bmGM) and segmented DTR masks were finally brought back into the subject space for PET signal extraction, using individual deformation fields.

PET acquisition and pre-processing

Tau-PET imaging was performed with radiotracer $^{[18F]}$ THK5351 on an ECAT EXACT+ HR scanner (Siemens, Erlangen, Germany). Participants received a single dose in an antecubital vein (target dose 185 MBq). A transmission scan was acquired first and dynamic image acquisitions started immediately after injection, consisting in 32 frames with increasing time duration. PET images were reconstructed using filtered back-projection algorithm including corrections for measured attenuation, dead time, random events, and scatter using standard software (ECAT 7.1, Siemens/CTI, Knoxville, TN, USA). For each individual, an average PET image was created using the 4 frames corresponding to the time window between 40 and 60 min (Lockhart et al., 2016). Averaged PET images were manually reoriented and automatically coregistered to the structural MT map. Subject space bmGM and segmented DTR masks were then applied to retrieve regional mean radiotracer uptake values. Standardized uptake value ratio (SUVR) was calculated using the cerebellum grey matter as the reference region (Ishiki et al., 2018). Mean $^{[18F]}$ THK5351 SUVR was 1.43 ± 0.11 over the whole brain, 1.97 ± 0.20 in the brainstem monoaminergic grey matter, and 2.27 ± 0.23 in the DTR mask.

While first generation tau protein radiotracers, including $^{[18F]}$ THK5351, have been widely studied and proved to have high affinity and selectivity *in vitro* (Lemoine et al., 2017), some criticisms were addressed towards their off-binding to monoamine oxidase B (MAO-B) *in vivo*, particularly over the basal ganglia (Chiotis et al., 2018). However, as investigated in a recent comparative analysis of first and second generation radiotracers, this off-target binding may be common to all first generation radiotracers and, to a lesser extent, also to second generation radiotracers (Murugan et al., 2019).

Tau co-localization analysis

Spatial processing of PET images was achieved with the ‘standard pipeline’ module in hMRI toolbox. Whole-brain $^{[18F]}$ THK5351 SUVR maps were diffeomorphically registered to the study-specific template using individual deformation fields, warped into the MNI space, and tissue-specific smoothing (full width at half-maximum of 6 mm isotropic) was applied. Voxel-based quantification (VBQ) analyses were carried out on these images in SPM12 in order to highlight brain regions which correlate with tau deposits in the DTR. Statistical modeling consisted in a multiple linear regression model including four regressors (*i.e.* demographic variables of age, sex, education, and DTR mean $^{[18F]}$ THK5351 SUVR). One-

tailed t tests were used to identify voxels displaying increased ^{18}F THK5351 SUVR associated with higher values in the DTR. Statistical threshold was set at $p < 0.05$ after family-wise error correction for multiple comparisons ($p_{\text{FWE-corrected}}$) voxel-wise for the whole-brain. Grey matter and white matter tissue classes were analyzed separately using explicit masks defining voxels belonging to each tissue class. These masks were generated using the across-subjects average of smoothed Jacobian-modulated tissue probability maps in MNI space, with a minimum probability threshold of 20%, and voxels were assigned to the tissue class for which the probability was maximal. For both VBQ analyses, DTR area was further masked to avoid redundancy.

DWI acquisition and processing

Diffusion-weighted imaging was performed using a multi-shell diffusion scheme (Caruyer, Lenglet, Sapiro, & Deriche, 2013). Data were acquired using a spin-echo echo-planar imaging (SE-EPI) sequence with 2 mm isotropic spatial resolution. Acquisition parameters were TR = 7900 ms, TE = 69 ms, 70 transverse slices with a slice thickness of 2 mm, in-plane resolution 2x2 mm² (FoV = 192x216 mm², matrix = 96x106), and acceleration factor 2, bandwidth per pixel = 2170 Hz/Px, and b-values of 650, 1000 and 2000 s/mm² with a number of diffusion sampling directions of 15, 30, and 60, respectively. The set of diffusion directions was created using electrostatic repulsion (Slater et al., 2019). This multi-shell diffusion-weighted imaging scheme included 118 volumes, of which the first volume was discarded to avoid T1 saturation effect. The remaining 117 volumes correspond to a total of 105 diffusion-weighted images with $b > 0$, interleaved with 12 $b = 0$ images. Susceptibility-induced and eddy current-induced distortions, as well as movements, were estimated and corrected using ‘topup’ (Andersson, Skare, & Ashburner, 2003) and ‘eddy’ (Andersson & Sotiropoulos, 2016) in FSL 5.0.9 (<https://fsl.fmrib.ox.ac.uk/fsl/fslwiki>). Diffusion directions were rotated to account for subject’s movement. The estimation of susceptibility-induced and eddy current-induced distortions relied on the acquisition of 5 additional $b = 0$ volumes with the same acquisition parameters as above but inverted phase encoding direction (Andersson et al., 2003).

Tractography analysis

Tractography analyses were performed in DSI Studio (July 9th build, <http://dsi-studio.labsolver.org>). A connectometry database was first created based on 61 diffusion MRI scans. The b-table (containing b-values and rotated b-vectors) was checked by an automatic

quality control routine to ensure its accuracy (Schilling et al., 2019). The diffusion data were reconstructed in the MNI space using q-space diffeomorphic reconstruction (Yeh & Tseng, 2011), a model-free method which computes the orientational distribution of the density of diffusing water in a study-specific standard space (Yeh, Wedeen, & Tseng, 2010). A deterministic fiber tracking algorithm (Yeh, Verstynen, Wang, Fernández-Miranda, & Tseng, 2013) was used with DSI Studio default tracking parameters (qa threshold of 0.0260504, angular threshold randomly selected from 15 degrees to 90 degrees, step size randomly selected from 0.5 voxel to 1.5 voxels, track length between 30 and 300 mm). For each hemisphere, 10000 tracts were generated with an anatomy prior from the Human Connectome Project (HCP-)842 tractography atlas (Yeh et al., 2018) corresponding to the fronto-pontine, parieto-pontine, temporo-pontine, and occipito-pontine tracts. Tracking routine was further restricted using our MRI-based segmented DTR mask as a ROI in order to assess cortico-DTR pathways.

The literature shows that both structural and functional connectivity may underlie tau propagation and influence on remote brain areas (Adams, Maass, Harrison, Baker, & Jagust, 2019; Pooler et al., 2013). We therefore focused our statistical analyses on quantitative anisotropy because it reflects both the structural and functional integrity of white matter tracts. In addition, while traditional diffusivity indices, such as fractional anisotropy, are recommended to detect structural alteration in pathological conditions, quantitative anisotropy is a DWI metric of density more sensitive to inter-individual differences in healthy individuals (Yeh et al., 2016). Mean quantitative anisotropy values were extracted over each cortico-DTR tracts bundles in a lobe-wise approach (*i.e.* fronto-DTR, parieto-DTR, temporo-DTR, and occipito-DTR) for each hemisphere and were used in regression analyses.

Statistical analysis

Statistical analyses were performed using Pearson's correlations and Generalized Linear Mixed Models (GLMMs) in SAS 9.4 (SAS Institute, Cary, NC, USA). Pearson's correlations were used as an assessment of potential association before computing GLMM which accounted for possible biases and covariate influences. Dependent variable distribution was first determined using 'allfitdist' function (developed by Mike Sheppard) in MATLAB2013a and GLMMs were adjusted accordingly. TMS-EEG stimulation parameters of applied electric field and distance between hotspot and recording electrode were included as covariates in GLMM statistical models with cortical excitability measures as dependent

variables. All GLMMs were adjusted for demographic variables of age, sex and education. Post-hoc tests were performed using differences of least squares means, and p -values were adjusted for multiple comparisons with Tukey's procedure. Statistical significance was set at $p < .05$. Statistical trend was defined as $p < .10$. Degrees of freedom were estimated using Kenward-Roger's correction. Semi-partial R^2 ($R^2_{\beta^*}$) values were computed to estimate the effect sizes of significant fixed effects and statistical trends in all GLMMs (Jaeger, Edwards, Das, & Sen, 2017b).

Results

Our analysis first focused on the link between cortical excitability, as assessed with TMS-EEG over the frontal cortex (TMS-evoked EEG potential – TEP), and tau burden in the brainstem monoaminergic grey matter (bmGM), measured with PET. We found a significant and positive association between TEP slope and bmGM mean $^{[18F]}$ THK5351 SUVR both in a simple correlation and after adjusting for TMS-EEG stimulation parameters and demographic variables (Pearson's correlation: $r = .32$, $p = .01$; GLMM: $F_{1,58} = 6.48$, $p = .01$, $R^2_{\beta^*} = .10$; **Fig. II.2A, Table II.2**). We then reduced the automatically-extracted bmGM region to a dorsal meso-pontine tegmentum region (DTR) including the LC (though not exclusively). The analysis revealed a similar positive association between TEP slope and DTR mean $^{[18F]}$ THK5351 SUVR, although as a statistical trend (Pearson's correlation: $r = .22$, $p = .08$; GLMM: $F_{1,58} = 3.23$, $p = .08$, $R^2_{\beta^*} = .05$; **Fig. II.2B, Table II.2**). The significant association between bmGM and cortical excitability may therefore arise at least in part from a dorsal portion of the tegmentum including the LC.

We extracted $^{[18F]}$ THK5351 SUVR over regions included in Braak stages 1-2 of tau pathology (*i.e.* hippocampus and entorhinal cortex) and in the late-affected isocortical areas from Braak stages 5-6 (*i.e.* widespread set of regions covering the frontal, parietal, occipital, and temporal cortices). We confirmed that mean $^{[18F]}$ THK5351 SUVR was superior in the DTR relative to the Braak stages 5-6 areas, but it was not significantly different from tau measure in the hippocampus/entorhinal cortex (GLMM including age, sex, education; main effect of brain region: $F_{3,192} = 1111.39$, $p < .0001$, $R^2_{\beta^*} = .95$; DTR > Braak 5-6, $p_{\text{adj}} < .0001$; **Fig. II.3A**). In addition, $^{[18F]}$ THK5351 SUVR was significantly inferior Braak stages 5-6 areas when compared to Braak stages 1-2 regions ($p_{\text{adj}} < .0001$). Voxel-based quantification (VBQ) analyses further revealed that DTR $^{[18F]}$ THK5351 SUVR was positively correlated with $^{[18F]}$ THK5351 SUVR in the hippocampus and entorhinal cortex, but also in the white matter

tracts connecting the brainstem to the medial temporal lobe (**Fig. II.3B, Table II.3**). These findings are in line with the literature showing that medial temporal lobe tau deposits follow brainstem tau lesions before tau accumulation is detected in higher associative cortical areas (Braak & Del Tredici, 2015).

Given the relative young age of our sample, we anticipated that tau NFT burden would be too low in the cortex to be associated with altered brain function. Accordingly, we found no significant association between TEP slope and mean $^{[18F]}$ THK5351 SUVR in late-affected isocortical brain areas (Braak stages 5-6; Pearson's correlation: $r = .09$, $p = .48$; GLMM: $F_{1,58} = 0.71$, $p = .40$; **Fig. II.2D**). We further found no significant association between hippocampal/entorhinal cortex $^{[18F]}$ THK5351 SUVR and TEP slope (Braak stages 1-2; Pearson's correlation: $r = .13$, $p = .32$; GLMM: $F_{1,58} = 1.85$, $p = .18$; **Fig. II.2C**). These results support the validity of our PET values and suggest that, in our sample, the association between cortical hyper-excitability and PET tau NFT assessment is specific to the brainstem.

We finally considered quantitative anisotropy to explore potential tractography correlates of cortical hyper-excitability assessed over the frontal cortex and DTR tau NFT burden. Given the wide projections of the LC to the cortex, we considered the integrity of white matter tracts linking the DTR and the entire cortex in a lobe-wise approach. We found a significant and positive association between TEP slope and quantitative anisotropy in fronto-DTR tracts, both bilaterally (Pearson's correlation: $r = .27$, $p = .03$; GLMM: $F_{1,58} = 6.31$, $p = .02$, $R^2_{\beta^*} = 0.09$, **Fig. II.4, Table II.4**) and when considering only the left hemisphere (*i.e.* the TMS target area in 97% of the participants; Pearson's correlation: $r = .31$, $p = .01$; GLMM: $F_{1,58} = 7.19$, $p = 0.01$, $R^2_{\beta^*} = 0.11$). These relationships seemed specific to fronto-DTR tracts as they were not significant when considering other cortico-DTR pathways (*i.e.* parieto-DTR, temporo-DTR, occipital-DTR; Pearson's correlations: $r < .09$, $p > .49$; GLMMs: $F_{1,58} < 2.44$, $p \geq .12$). This suggests that higher frontal cortical excitability is associated with increased diffusion density in the white matter tracts connecting the frontal cortex and DTR. In contrast, we found no significant association between tau NFT PET value in the DTR and quantitative anisotropy across all cortico-DTR pathways (Pearson's correlations: $r < .13$, $p \geq .30$; GLMMs: $F_{1,60} < 0.78$, $p \geq .38$).

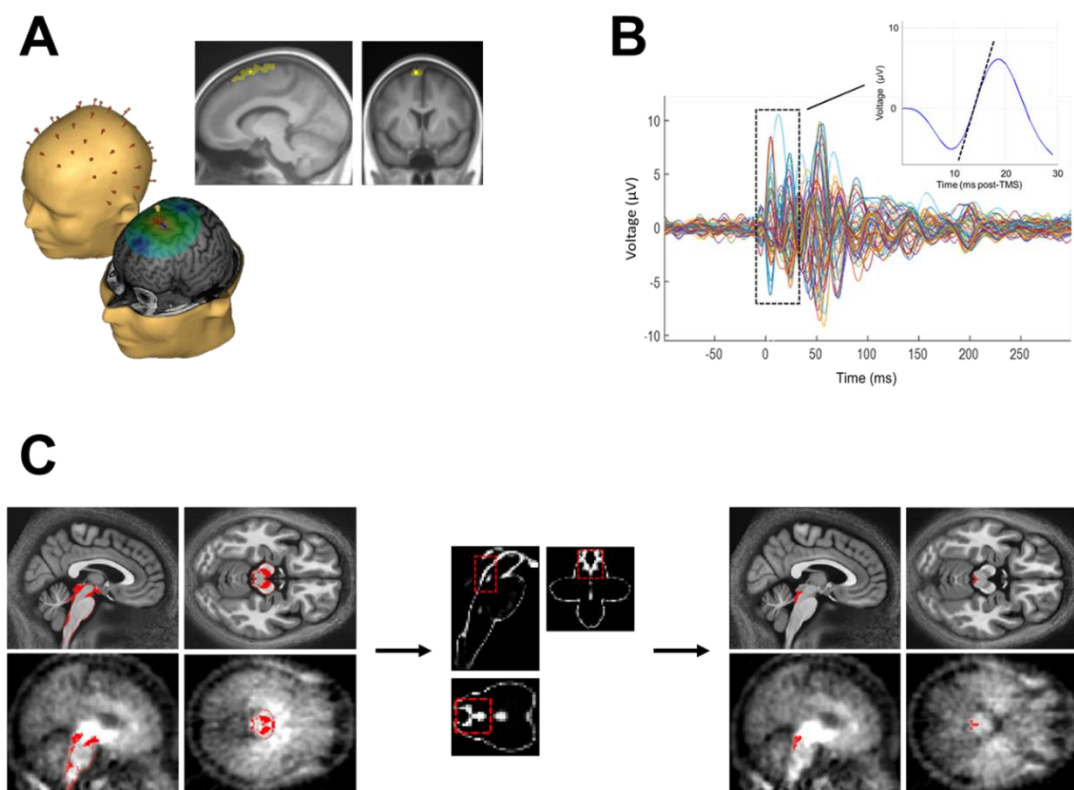


Figure II.1. Cortical excitability assessment and brainstem SUVR extraction.

A) Cortical excitability over the frontal cortex was assessed using neuronavigation-based TMS coupled to EEG. TMS-EEG target was located in the superior frontal gyrus. Darker and brighter yellow area represents the range of stimulation targets across participants projected onto the averaged normalized T1 structural volume and the median TMS-EEG stimulation hotspot over the sample, respectively.

B) Butterfly plot of TMS-evoked EEG response over the 60 electrodes (-100 ms pre-TMS to 300 ms post-TMS; average of ~250 trials). Cortical excitability was computed as the slope ($\mu\text{V}/\text{ms}$, dotted line on inset) of the first component of the TEP response at the electrode closest to the stimulation hotspot.

C) Automatic brainstem segmentation methods were used to extract ^{18}F THK5351 SUVR in the brainstem monoaminergic grey matter (bmGM; left panels). A dorsal meso-pontine tegmentum region (DTR; right panels), including the locus coeruleus (LC), was manually delineated based on the normalized smoothed bmGM segmented tissues, and the DTR mask was applied to native PET images to extract individual regional tau protein burden.

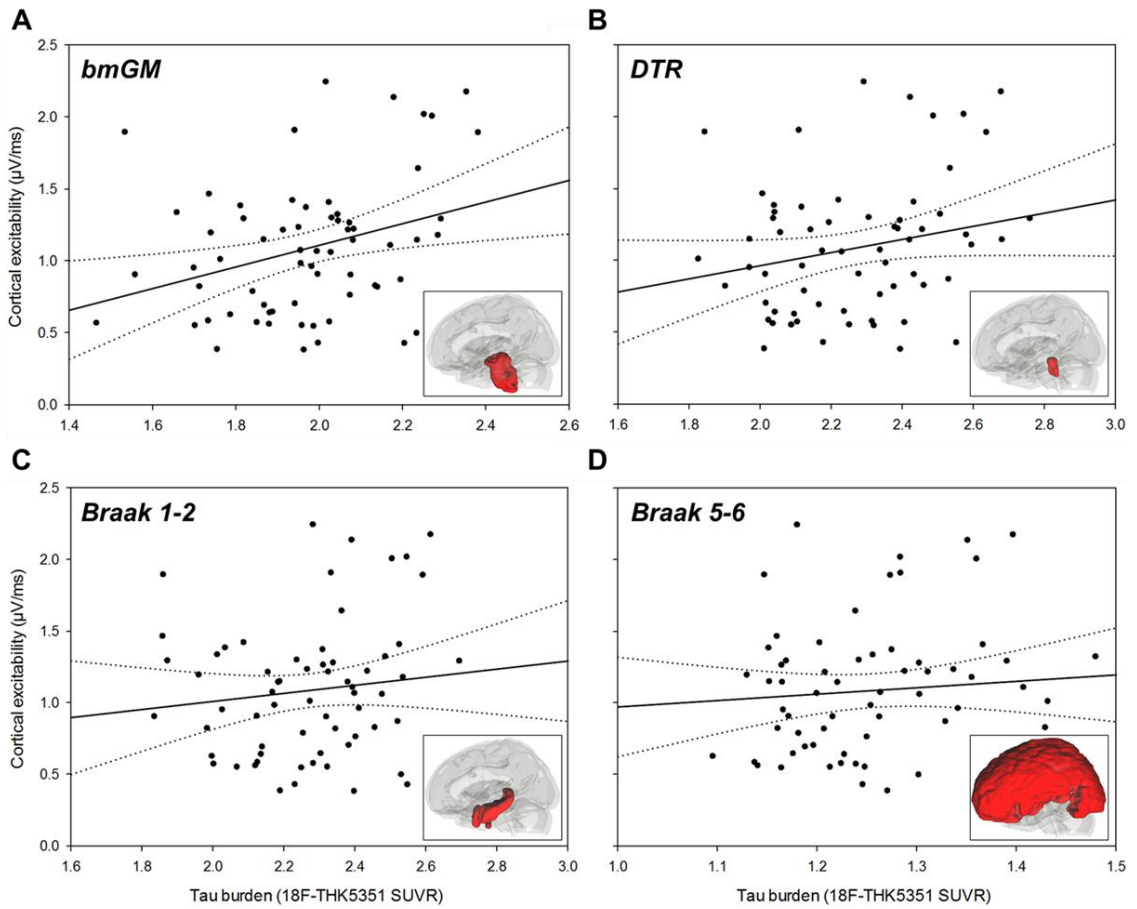


Figure II.2. Associations between tau protein burden and cortical excitability.

A) Significant and positive association between cortical excitability values and mean ^{18}F THK5351 standardized uptake value ratio (SUVR) in the brainstem monoaminergic grey matter (bmGM; Pearson's correlation: $r = .32$; $p = .01$; GLMM: $F_{1,58} = 6.48$, $p = .01$, $R^2_{\beta^*} = 0.10$).

B) Statistical trend towards a significant and positive association between cortical excitability and mean ^{18}F THK5351 SUVR in a dorsal tegmentum region (DTR), including notably the LC (Pearson's correlation: $r = .22$; $p = .08$; GLMM: $F_{1,58} = 3.23$, $p = .08$).

C) No significant association between cortical excitability and mean ^{18}F THK5351 SUVR in the entorhinal cortex and hippocampus (Braak stages 1-2; Pearson's correlation: $r = .10$; $p = .43$; GLMM: $F_{1,58} = 1.42$, $p = .24$).

D) No significant association between cortical excitability values and mean ^{18}F THK5351 SUVR in late-affected isocortical brain areas (Braak stages 5-6; Pearson's correlation: $r = .09$; $p = .48$; GLMM: $F_{1,58} = 0.71$, $p = .40$).

Simple regressions are displayed and full GLMMs outputs are reported in **Table II.2**. Dotted lines represent 95% confidence interval for these simple regressions. All GLMMs included age, sex, education and TMS-EEG acquisition parameters (mean estimated electric field induced by TMS pulse and distance between considered electrode and stimulated cortical hotspot) as covariates.

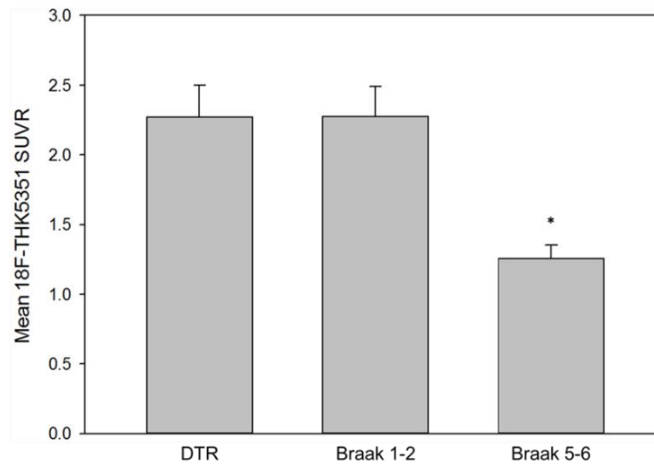
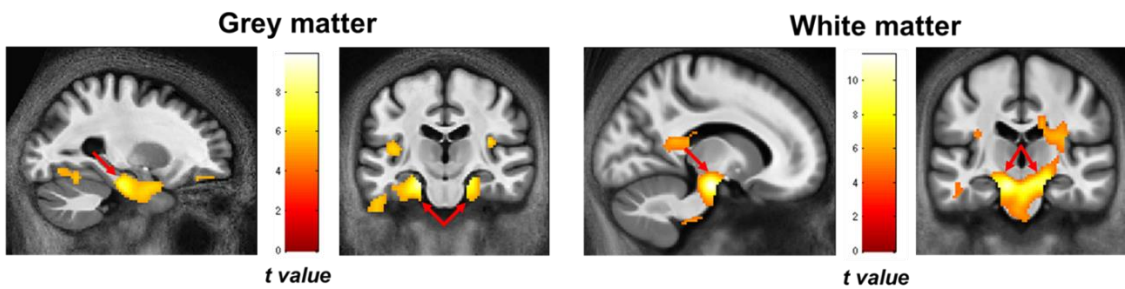
A**B**

Figure II.3. Regional tau protein burden and VBQ analysis.

A) ^{18}F THK5351 SUVR in the dorsal tegmentum region (DTR) is not significantly different from Braak stages 1-2 areas (hippocampus/entorhinal cortex). Tau burden in late-affected isocortical areas from Braak stages 5-6 is significantly lower than all other considered areas. $*p_{\text{adj}} < .001$.

B) VBQ analysis showed that increased ^{18}F THK5351 SUVR in the DTR is associated with increased ^{18}F THK5351 SUVR in hippocampus and entorhinal cortex grey matter ($p_{\text{FWE-corrected}} < .001$; red arrows, left panels), as well as in the brainstem ascending pathways linking the medial temporal lobe ($p_{\text{FWE-corrected}} < .001$; red arrows, right panels). Detailed clusters results are presented in **Table II.3**.

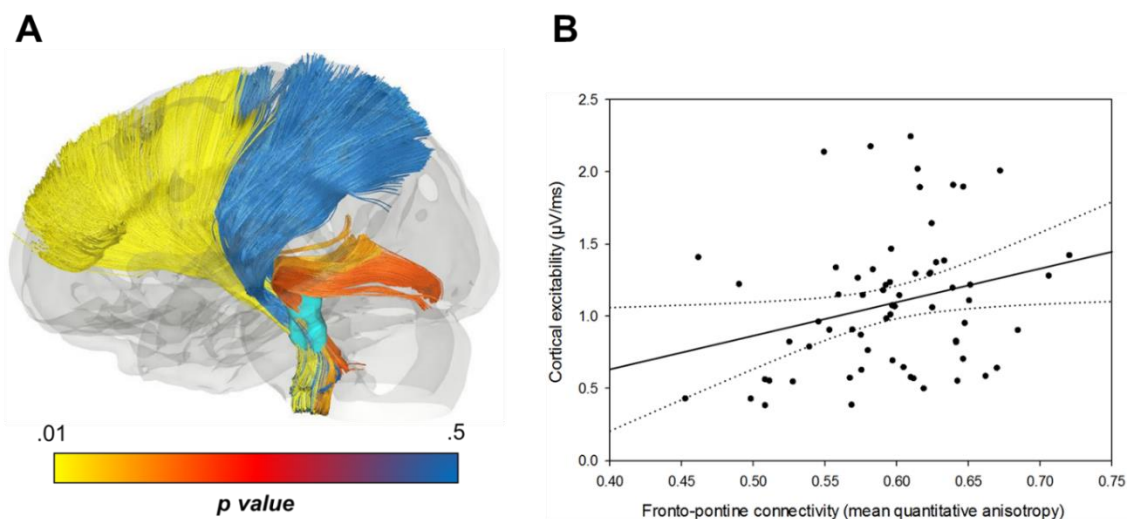


Figure II.4. Cortico-pontine tractography analysis.

A) Lobe-wise tractography-based analyses revealed that, among cortico-pontine pathways, frontal cortical excitability is positively associated with mean quantitative anisotropy in fronto-pontine tracts (yellow; the horizontal color bar correspond to p -values for each lobe). The cyan blue area corresponds to the dorsal tegmentum ROI used in the cortico-pontine tracking routine in DSI studio software.

B) Average quantitative anisotropy values in fronto-pontine tracts showed a significant linear association with frontal cortex excitability, as assessed through TEP slope (Pearson's correlation: $r = .27$, $p = .03$; GLMM: $F_{1,58} = 6.31$, $p = .01$, $R^2_{\beta*} = 0.10$). GLMM included age, sex, education, and TMS-EEG acquisition parameters (mean estimated electric field induced by TMS pulse and distance between considered electrode and stimulated cortical hotspot) as covariates.

Table II.1. Sample characteristics (mean $\pm$ SD).

	N = 65
Sex	46F/19M
Age (years)	59.6 $\pm$ 5.3
Education (years)	15.4 $\pm$ 3.1
Right-handed (%)	97
Ethnicity	Caucasian
Dementia Rating Scale	142.3 $\pm$ 2
Body mass index (Kg/m ²)	24.4 $\pm$ 2.7
Whole-brain [¹⁸ F]THK5351 (SUVR)	1.43 $\pm$ 0.11
Brainstem monoaminergic grey matter [¹⁸ F]THK5351 (SUVR)	1.97 $\pm$ 0.20
Dorsal tegmentum area [¹⁸ F]THK5351 (SUVR)	2.27 $\pm$ 0.23
Hippocampal/entorhinal cortex [¹⁸ F]THK5351 (SUVR)	2.28 $\pm$ 0.21
TMS-evoked cortical excitability (μ V/ms)	1.09 $\pm$ 0.48
TMS applied electric field (V/m)	114.7 $\pm$ 15
Recording electrode distance from TMS hotspot (mm)	45.4 $\pm$ 10.5

F = female; M = male; SUVR= standardized uptake value ratio.

Table II.2. GLMM outputs of the associations between cortical excitability values and mean ^{18}F THK5351 SUVR in the brainstem monoaminergic grey matter (bmGM) and a dorsal tegmentum region (DTR).

	<i>bmGM</i>	<i>DTR</i>
Mean ^{18}F THK5351 SUVR	$F_{1,58} = 6.48$ $p = 0.01$ $R^2_{\beta^*} = 0.10$	$F_{1,58} = 3.23$ $p = 0.08$ $R^2_{\beta^*} = 0.05$
Age	$F_{1,58} = 3.06$ $p = 0.09$ $R^2_{\beta^*} = 0.05$	$F_{1,58} = 3.38$ $p = 0.07$ $R^2_{\beta^*} = 0.06$
Sex	$F_{1,58} = 0.03$ $p = 0.87$	$F_{1,58} = 0.10$ $p = 0.76$
Education	$F_{1,58} = 3.49$ $p = 0.07$ $R^2_{\beta^*} = 0.06$	$F_{1,58} = 2.90$ $p = 0.09$ $R^2_{\beta^*} = 0.05$
Electric field	$F_{1,58} = 1.77$ $p = 0.19$	$F_{1,58} = 1.66$ $p = 0.20$
Record. elect. dist	$F_{1,58} = 3.72$ $p = 0.06$ $R^2_{\beta^*} = 0.06$	$F_{1,58} = 3.96$ $p = 0.05$ $R^2_{\beta^*} = 0.06$

TEP slope was used as the dependent variable, as a measure of cortical excitability. $R^2_{\beta^*}$ values correspond to semi-partial R^2 in GLMMs, and are reported for all significant associations and statistical trends.

Table II.3. Cluster results for grey and white matter VBQ analyses of ^[18F]THK5351 SUVR with increased tau burden in the dorsal tegmentum region mask, after adjusting for regressors of age, sex, and education.

Cluster extent (voxels)	Localization label (peak level)	Peak coordinates (XYZ, mm)	<i>t</i> statistics (peak level, DF=1,60)	<i>p</i> _{FWE-corrected} (peak level)
Grey matter				
2789	Left entorhinal cortex	-16 -18 -18	9.69	< .001
	Left fusiform gyrus	-38 -58 -14	8.62	< .001
	Left anterior temporal cortex	-32 -2 -30	6.41	< .001
1215	Right entorhinal cortex	16 -16 -18	8.14	< .001
	Right anterior hippocampus	20 -22 -14	7.67	< .001
	Mammillary bodies	6 -16 -14	7.21	< .001
1552	Right retrosplenial cortex	10 -44 6	7.54	< .001
	Right fusiform gyrus	32 -68 -12	7.21	< .001
	Right inferior temporal cortex	38 -60 -14	7.02	< .001
242	Right insula	42 -16 12	6.83	< .001
221	Left insula	-32 22 10	6.26	.001
219	Left insula	-36 -14 16	5.41	.001
White matter				
11237	Cortico-pontine tract	0 -24 -24	11.47	< .001
	Left cortico-pontine tract	-14 -16 -18	11.40	< .001
	Right cortico-pontine tract	16 -16 -16	10.29	< .001
389	Right Inferior longitudinal fasciculus	44 0 -26	6.30	< .001
Voxel size = 2 mm ³ ; DF = degrees of freedom.				

Table II.4. GLMMs outputs of the associations between cortical excitability and lobe-wise tractography-based mean quantitative anisotropy values.

	<i>Fronto-DTR</i>	<i>Parieto-DTR</i>	<i>Occipito-DTR</i>	<i>Temporo-DTR</i>
Mean quantitative anisotropy	$F_{1,58} = 6.31$ $p = .01$ $R^2_{\beta^*} = 0.10$	$F_{1,58} = 0.01$ $p = .94$	$F_{1,58} = 2.14$ $p = .15$	$F_{1,58} = 1.98$ $p = 0.17$
Age	$F_{1,58} = 2.75$ $p = .10$	$F_{1,58} = 2.83$ $p = .10$	$F_{1,58} = 2.60$ $p = .11$	$F_{1,58} = 2.34$ $p = .13$
Sex	$F_{1,58} = 0.87$ $p = .36$	$F_{1,58} = 0.01$ $p = .92$	$F_{1,58} = 0.52$ $p = .47$	$F_{1,58} = 0.65$ $p = .42$
Education	$F_{1,58} = 1.75$ $p = .19$	$F_{1,58} = 1.90$ $p = .17$	$F_{1,58} = 2.75$ $p = .10$	$F_{1,58} = 2.58$ $p = .11$
Electric field	$F_{1,58} = 1.66$ $p = .20$	$F_{1,58} = 1.01$ $p = .32$	$F_{1,58} = 1.33$ $p = .25$	$F_{1,58} = 1.40$ $p = .24$
Record. elect. dist	$F_{1,58} = 2.93$ $p = .09$ $R^2_{\beta^*} = 0.05$	$F_{1,58} = 4.51$ $p = .04$ $R^2_{\beta^*} = 0.07$	$F_{1,58} = 3.12$ $p = .08$ $R^2_{\beta^*} = 0.05$	$F_{1,58} = 3.64$ $p = .06$ $R^2_{\beta^*} = 0.06$

TEP slope was used as the dependent variable, as a measure of cortical excitability. $R^2_{\beta^*}$ values correspond to semi-partial R^2 in GLMMs, and are reported for all significant associations and statistical trends.

Discussion

We provide evidence that tau NFT accumulation in a compartment of the brainstem including monoaminergic neurons is associated with increased cortical excitability in cognitively normal individuals aged 50 to 70 y. This association was not significant when considering tau burden measured over medial temporal or associative cortical regions. When reducing the bmGM to a small region of the dorsal tegmentum including notably the LC, the association remained as a statistical trend. In line with human *post-mortem* studies (Braak, Thal, Ghebremedhin, & Tredici, 2011), we further demonstrate that tau burden in the DTR is superior to tau measure in associative cortical areas but is not significantly different from the Braak stages 1-2 regions, *i.e.* the hippocampus and entorhinal cortex. Together, these findings support that increased cortical excitability is related to the presence of tau aggregates, specifically in the brainstem. We also show that cortical excitability is associated with quantitative anisotropy of white matter tracts connecting the DTR to the frontal cortex, suggesting that the integrity of these tracts may be part of the observed relationship between brainstem tau burden and cortical excitability.

Increased cortical excitability was previously reported in MCI and AD patients, *i.e.* in the presence of cognitive symptoms (Ferreri et al., 2011; Olazarán et al., 2010; Trebbastoni et al., 2016). Our results show that cortical hyper-excitability is present during the preclinical phase of AD, *i.e.* over an asymptomatic period during which AD-related pathophysiological processes evolve and may lead to subsequent symptomatic AD in some individuals. A rationale often proposed to account for the increased cortical excitability in MCI and first stages AD patients postulates that it represents a compensatory mechanisms to counteract synaptic dysfunction and tau NFT accumulation within neuronal networks (D'Amelio & Rossini, 2012). A recent computational model of neuronal dynamics in AD reported that the most efficient intervention to counter network dysfunction and enhance its functional survivability was a selective increase in excitability of excitatory neurons (de Haan, van Straaten, Gouw, & Stam, 2017). This compensatory phenomenon may in turn favor pathological progression as neuronal hyper-activity increases amyloid-beta and tau release (Bero et al., 2011; Yamada et al., 2014). A vicious circle would therefore take place up to a point where A β and tau burden and neurodegeneration would be too high to sustain increased excitability (Holth et al., 2013; Kellner, Menkes-Caspi, Beker, & Stern, 2014; Palop & Mucke, 2010). This would imply an inverted U-shape relationship between cortical excitability and

network integrity over AD pathophysiological processes, with progressive increase in cortical excitability compensating tau-driven synaptic dysfunction early in the process, followed by decreased excitability in more advanced stages of tau deposition.

The fact that cortical excitability was associated with tau PET measure in a brainstem region including monoaminergic neurons and also, as a statistical trend, when focusing on a dorsal part of the tegmentum, supports that the association arises, at least in part, from the LC, or from nearby monoaminergic (e.g. raphe) and non-monoaminergic nuclei likely included in the dorsal tegmentum mask we used, and part of the ascending reticular activating system. Higher resolution PET and MRI data geared towards isolating the neuromelanin dependent signal of the LC (Clewett et al., 2016) are required to truly test this assumption. Previous animal studies reported that cortical excitability increased as a function of tau burden in the hippocampal region or reported reduced cortical excitability following genetic manipulation reducing overall endogenous tau levels (DeVos et al., 2013; Hall et al., 2015; Holth et al., 2013; Roberson et al., 2007). Our results may therefore not be specific to the brainstem (and its monoaminergic neurons) but may be in relation to the presence of tau NFT *per se*. Cortical excitability across the different steps of the stereotypical outward progression of tau NFTs would have to be monitored to resolve this issue. We nevertheless provide the first functional correlates of tau PET measures in the brainstem in healthy individuals with tau spread limited to its early stages.

In contrast to fractional anisotropy which, for a given track, is a voxel-based index directly linked to the structural integrity of axons (Basser & Pierpaoli, 1996), quantitative anisotropy is related to both integrity of the track, *i.e.* the ability of water molecules to move in a constrained direction, and to the density of water molecules transiting within this constrained structure (Yeh et al., 2013). Quantitative anisotropy is therefore a composite measure reflecting both structural and functional aspects of axonal function. It has been proposed to be very sensitive to inter-individual variations in connectivity in healthy individuals devoid of neurological disorders, such as those included in our sample (Yeh et al., 2016). Our exploratory findings point towards a role of the connectivity between frontal and dorsal pontine structures in the association between frontal cortex excitability and DTR tau measurements. A stronger connectivity could facilitate information flow between the frontal cortex and the brainstem nuclei part of the ascending reticular activating system, including the LC, resulting in increased cortical excitability, tau release, or both.

Given the cross-sectional nature of our study, we cannot infer about causality, and mechanisms underlying our findings remain elusive. In a first scenario, we propose that the progressive accumulation of misfolded tau protein in the brainstem, and maybe the LC, triggers increased cortical excitability as a compensatory mean to face tau NFT accumulation, potentially through increased connectivity of the fronto-pontine tracts. Whole-cell patch-clamp recordings in a mouse model of progressive tauopathy found that frontal cortical neurons exhibit electrophysiological alterations including a more depolarized resting membrane potential (Crimins, Rocher, & Luebke, 2012), which would result in increased cortical excitability. In addition, altered excitation/inhibition balance and subsequent hyper-excitability may be a consequence of tau accumulation through tau-dependent inhibitory interneurons depletion (Andrews-Zwilling et al., 2010). Although these observations lend support to our first scenario, they were never focused on brainstem tau burden. One cannot exclude therefore a second scenario, where cortex excitability would favor tau production, release, and aggregation in the brainstem. In this scenario, frontal cortex excitability could increase in response to environmental factors (e.g. anxiety, lack of sleep), while fronto-pontine increased connectivity could either contribute to tau release or come as a consequence of increased cortical excitability.

Our experiment bears several limitations, the most important being the use of [¹⁸F]THK5351, a first generation PET marker of tau NFT. [¹⁸F]THK5351 has been reported to bind to MAO-B, a marker of neuroinflammation, in addition to tau NFT. This non-specific binding is however most pronounced over the basal ganglia, and is also found, although to a lesser extent, in second generation tau radiotracers. However, the progressive decrease in [¹⁸F]THK5351 uptake observed in our data from the DTR to the associative cortical areas supports that it is, at least, a partial marker of tau NFT. Interestingly, neuroinflammatory and microglial dysfunction mechanisms have been proposed to result from tau-mediated neurodegeneration of LC neurons, potentially through norepinephrine depletion (Heneka et al., 2010; Laurent, Buée, & Blum, 2018; Satoh & Iijima, 2019). Therefore, although it is unlikely to fully explain our results, we cannot exclude that neuroinflammation, and potentially tau-induced neuroinflammation, contributed to our findings. Furthermore, we measured cortical excitability exclusively over the frontal cortex. While this area allows for minimally artefacted data, probing cortical excitability over different parts of the cortex may reveal a broader relationship between early tau aggregates and alterations of neuronal function. Another limitation of our study is that it only includes healthy individuals with

limited tau NFT spread. Individuals with more advanced tau pathology, MCI and early AD patients would have provided further characterization of the link between tau and cortical excitability. Yet, individuals included in our study sample were thoroughly screened for their health and cognitive status, reducing the probability of biases arising from health conditions or AD comorbidities. In addition, individuals' sleep-wake history was carefully controlled prior to TMS-EEG data acquisition, and cortical excitability measures were acquired in the morning, after a fixed duration of wakefulness to account for the known changes in cortical excitability with prior wakefulness duration and time-of-day (Gaggioni et al., 2019; Huber et al., 2013; Ly et al., 2016). Furthermore, TMS-EEG allows to assess cortical excitability directly and reliably, while bypassing any sensory bias. Finally, we used state-of-the-art brainstem segmentation methods based on quantitative multiparametric imaging to automatically and systematically isolate brainstem monoaminergic neurons.

In conclusion, the present results point to brainstem tau NFT as a potential substrate for the somewhat paradoxical increased excitability in the early stages of AD neuropathology. These findings bring new insights into the interplay between cortical function and the first signs of tau aggregation in the brainstem. Furthermore, they suggest that cortical excitability could constitute a biomarker of early misfolded tau protein aggregates and of increased risk for AD.

Chapter III

Medial prefrontal cortex myelination is associated with sleep slow waves in healthy aging

This results section is in preparation for publication as a Research Article.

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Abstract

Aging is associated with alterations in sleep-wake regulation that have been directly associated with changes in brain structural integrity. In particular, the ability to generate slow oscillations during non-rapid eye movement (NREM) sleep decreases as early as in the 5th decade of life, predominantly over the frontal regions. While previous investigations have identified macrostructural brain correlates of the age-related decrement in sleep slow waves, their relationships with brain tissue microstructure remain poorly understood. Here, we recorded habitual sleep under electroencephalography (EEG) in 101 healthy older individuals (mean age = 59.44 ± 5.29 years; 68 women), and we quantified slow wave generation through the overnight cumulated power density in the delta band (0.75-4Hz) during NREM sleep. In addition, we used a quantitative multiparametric magnetic resonance imaging (qMRI) to assess myelin, water, and iron content in the medial prefrontal grey matter. We first found the expected age-related decrease in slow wave generation as well as in brain microstructural integrity. Strikingly, we found that a higher amount of overnight NREM sleep slow waves was associated with higher values of qMRI parameters related to tissue myelination. Our findings suggests that preserved myelin content in medial prefrontal grey matter may promote slow wave generation during NREM sleep in aging. Given the existing links between sleep characteristics and age-related cognitive decline, our findings may have implications for successful cognitive aging.

Introduction

Human aging is associated with marked changes in sleep and wakefulness regulation (Mander et al., 2017). With increasing age, on the one hand, the circadian signal favouring sleep or wakefulness over the 24h day is phase-advanced, leading to earlier timings of sleep and wakefulness onset, and its strength is reduced, contributing to the fragmentation of the sleep-wake cycle reflected in more awakenings during the night and the intrusion of naps during the day (Dijk, Duffy, & Czeisler, 2000; Duffy, Zitting, & Chinoy, 2015; Van Someren, 2000). On the other hand, sleep homeostasis processes, which build the need for sleep, are also affected as reflected by an age-related reduction in the ability to generate sleep slow waves, an electroencephalographic (EEG) gold standard marker of sleep pressure (Nir et al., 2011). These changes take place as early as in the 5th decade of life (Cajochen et al., 2006; Carrier et al., 2011). Furthermore, investigation of sleep slow wave generation is especially evident over the frontal lobe (Dubé et al., 2015; Murphy et al., 2009), which is both the most sensitive region to sleep-wake history (Schmidt et al., 2012) and the first to display a significant reduction in sleep slow waves density across the lifespan (Carrier et al., 2011; Mander et al., 2013).

Age-related changes in sleep-wake regulation have been associated with alteration in brain macrostructure and may predict future cognitive decline (Gagnon et al., 2019; Oosterman et al., 2009). Cross-sectional studies found a relationship between changes in grey matter integrity measures, such as cortical volume or thickness, and lower indices of self-reported or objectively measured EEG- and actigraphy-derived sleep-wake parameters (Carvalho et al., 2017; Dubé et al., 2015; A. S. P. Lim et al., 2016; Sanchez-Espinosa et al., 2014; Van Someren et al., 2018). In longitudinal protocols, these indices have been in turn reported to predict higher rates of brain structural deterioration, as measured through global and regional grey matter atrophy (Sexton et al., 2014; Spira et al., 2016) or ventricles expansion (Lo et al., 2014), suggesting bidirectional detrimental influences between alteration in brain macrostructure and impaired sleep quality (Ju et al., 2014; Van Egroo, Narbutas, Chylinski, Villar González, Maquet, Salmon, Bastin, et al., 2019).

Recent advances in magnetic resonance imaging (MRI) have provided new means to investigate brain microstructure, which is likely to be associated with age-related changes in sleep-wake regulation. Based on quantitative MRI measurements, it is now possible to perform so-called MRI-based *in vivo* histology (*hMRI*) to infer unbiased parameters related

to myelin, water, and iron content (Weiskopf et al., 2015). Among them, proxy measures of myelination are of particular interest, as myelin affects neural signal propagation. As a matter of fact, myelin has been reported to be closely related to sleep slow waves characteristics in childhood (Kurth et al., 2017), adolescence (Goldstone et al., 2018), and in early adulthood (Piantoni et al., 2013). However, little is known about this relationship in older individuals, when a widespread demyelination occurs, particularly over the frontal regions (Callaghan et al., 2014).

Here, we measured sleep slow waves in a sample of healthy older individuals aged 50 to 69 years during a night of habitual sleep under EEG following 7 days of regular sleep-wake schedule. We computed individual slow wave energy (SWE) which consists in the overnight cumulated power in the delta EEG frequency band (0.75-4 Hz) generated during NREM sleep. We acquired whole-brain quantitative maps using a multiparametric mapping (MPM) protocol (Draganski et al., 2011; Tabelow et al., 2019), and we extracted indices of grey matter density, magnetization transfer saturation, proton density, and longitudinal as well as effective transverse relaxation rates over a medial prefrontal region of interest. We hypothesized that grey matter density as well as magnetization transfer saturation, which most closely reflects myelin content (Callaghan et al., 2014; Schmierer et al., 2007), would be associated with both age and SWE.

Methods

Study design and participants

101 healthy older individuals (59.44 ± 5.29 years; 68 women) were enrolled for this study (**Table III.1**). They were all devoid of clinical symptoms of cognitive impairment (Dementia rating scale < 130 (S. Mattis, 1976); Mini mental state examination < 27). Other exclusion criteria were: body mass index ≤ 18 and ≥ 29 ; recent psychiatric history or severe brain trauma; addiction, chronic medication affecting the central nervous system; hypertension; smoking, excessive alcohol (> 14 units/week) or caffeine (> 5 cups/day) consumption; anxiety, as measured by the 21-item self-rated Beck Anxiety Inventory (BAI ≥ 17 (Beck, Epstein, et al., 1988)); depression, as assessed by the 21-item self-rated Beck Depression Inventory (BDI ≥ 17 (Beck, Steer, et al., 1988)). Participants with stable treatment (for > 6 months) for hypertension and/or hypothyroidism were included in the study. Participants with sleep apnea (apnea-hypopnea index ≥ 15 /hour) were excluded following an in-lab adaptation and screening night of polysomnography.

All participants gave their written informed consent, and received a financial compensation. This study was approved by the Ethics Committee of the Faculty of Medicine at the University of Liège, Belgium.

Magnetic resonance imaging acquisition

MRI data were acquired on a 3 Tesla whole-body Magnetom Prisma scanner (Siemens, Erlangen, Germany). Structural and quantitative maps of T1, T2*, proton density (PD) and magnetization transfer saturation (MT) were calculated from a multi-parameter protocol based on a 3D multi-echo fast low angle shot (FLASH) sequence (Weiskopf & Helms, 2008). Three co-localised 3D multi-echo FLASH data sets were acquired with predominantly proton density weighting (PDw: TR/FA=23.7 ms/6°), T1 weighting (T1w: TR/FA=18.7 ms/20°), and MT weighting (MTw: TR/FA=23.7 ms/6°; excitation preceded by an off-resonance Gaussian MT pulse of 5 ms duration, 220° nominal flip angle, 2 kHz frequency offset), with a voxel size of 1 mm³ isotropic. Total acquisition time was approximately 19 min. Two additional calibration sequences were acquired to correct for inhomogeneities in the RF transmit field (Lutti, Hutton, Finsterbusch, Helms, & Weiskopf, 2010).

Magnetic resonance imaging processing

Quantitative multi-parametric maps (MT, PD, R1, R2*; **Fig. III.1**) were generated with the hMRI toolbox (Tabelow et al., 2019) (<http://hmri.info>) embedded in SPM12 (www.fil.ion.ucl.ac.uk/spm) using MATLAB2013a (The Mathworks Inc., Natick, MA, USA). Spatial normalization of each of the four quantitative maps was performed using the 'standard pipeline' procedure in the hMRI toolbox, which consists in the following processing steps: segmentation of MT maps into individual tissue class maps of grey matter (GM), white matter (WM), and cerebrospinal fluid posterior probability using Unified Segmentation (Ashburner & Friston, 2005); diffeomorphic registration of quantitative maps to a study-specific template created with DARTEL, a non-linear diffeomorphic algorithm (Ashburner, 2007); Normalization to the Montreal Neurological Institute (MNI) space using DARTEL-estimated individual deformation fields; Smoothing of normalized quantitative maps using a tissue-specific weighting which accounts for the partial volume distribution of both GM and WM tissues in each voxel (Draganski et al., 2011), with a full-width at half maximum kernel of 6 mm isotropic.

Our ROI was defined as a set of medial prefrontal regions commonly involved in investigations of sleep slow waves (Dang-Vu et al., 2005, 2010; Mander et al., 2015, 2013; Saletin et al., 2013) (**Fig. III.2**). Based on regions from the Automated Anatomical Labeling atlas (Tzourio-Mazoyer et al., 2002), we built a composite mask including, bilaterally, medial prefrontal, medial orbital, medial orbito-frontal, rectus, and anterior cingulate cortices. Mean regional MT, PD, R1, and R2* signals were then computed by applying our ROI on normalized GM-weighted smoothed quantitative maps. In addition to hMRI parameters, we also extracted mean GM density in the ROI, based on Jacobian-modulated GM tissue probability maps.

Sleep assessment and spectral power analysis

As part of the screening process, participants first performed an in-lab adaptation and screening night to minimize the disrupting effect caused by sleeping in a novel environment (Tamaki et al., 2016), which might have otherwise affected the sleep parameters subsequently assessed during the baseline night. Then, for 7 days prior to the baseline night, participants followed a regular sleep-wake schedule (± 30 min), in agreement with their preferred bed and wake-up times. Compliance was verified using sleep diaries and wrist actigraphy (Actiwatch[®], Cambridge Neurotechnology, UK). Aside from the fixed sleep-wake schedule, participants were also instructed to abstain from unusually intense physical exercise for the last 3 days of fixed-schedule circadian entrainment. Their habitual sleep was then recorded in complete darkness under EEG (**Fig. III.1**). Sleep EEG data were acquired using N7000 amplifiers (EMBLA, Natus Medical Incorporated, Planegg, Germany). The electrode montage consisted of 11 EEG channels (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1, O2), 2 bipolar EOGs, and 2 bipolar EMGs. Scoring of baseline night in 30-s epochs was performed automatically using a validated algorithm (ASEEGA, PHYSIP, Paris, France) (Berthomier et al., 2007). An automatic detection algorithm with adapting thresholds was further applied to exclude artefacts and arousals from scored data (Coppieters 't Wallant et al., 2016). Power spectrum was computed for each channel using a Fourier transform on successive artefact-and-arousal-free 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 (0.75-4Hz) during all the NREM 2 (N2) and NREM 3 (N3) across 30s epochs, after adjusting for the number of artefacted 4-s windows in each 30-s epoch (Skorucak et al., 2018). SWE values in 30-min periods were further adjusted for the number of entire N2 and N3 corrupted epochs

(Skorucak et al., 2018). Overnight SWE was computed as the sum of SWE across all 30-min periods, and was used in our statistical analyses. As the frontal regions are most sensitive to sleep-wake history (Schmidt et al., 2012), SWE was extracted and averaged over the frontal derivations (F3, Fz, F4). Total time spent asleep was computed as the sum of all epochs scored as sleep, starting from the first N2 period until the end of the night.

Statistics

Statistical analyses were performed using Generalized Linear Mixed Models (GLMMs) in SAS 9.4 (SAS Institute, Cary, NC). Dependent variables distribution was first determined using “allfitdist” function in MATLAB and GLMMs were adjusted accordingly. GLMMs were adjusted for demographic variables of age, sex, and education, as well as total sleep time. Statistical significance was set at $p < 0.05$. Degrees of freedom in GLMMs were estimated using Kenward-Roger’s correction. Semi-partial R^2 ($R^2_{\beta^*}$) values were computed to estimate the effect size of significant fixed effects in all GLMMs (Jaeger et al., 2017a).

Results

Age-related changes in brain (micro-)structure

We first assessed the effect of age on quantitative parameters in the frontal ROI, using a set of GLMMs adjusted for sex and education. We found that age was significantly and negatively associated with MT ($F_{1,97} = 10.56, p = .002, R^2_{\beta^*} = .10$) and PD ($F_{1,97} = 9.46, p = .003, R^2_{\beta^*} = .09$) indices (**Fig. III.3A-B**). In addition, age was associated with an expected decrease in GM density ($F_{1,97} = 7.51, p = .008, R^2_{\beta^*} = .07$; **Fig. III.3C**). By contrast, no significant relationship with age was found for R1 ($F_{1,97} = 2.84, p = .10$) and R2* ($F_{1,97} = 0.47, p = .49$) measures (**Fig. III.3D-E**).

Sex- and age-related decrease in slow wave energy

Across our sample, mean total sleep time during the night was 391.81 ± 45.38 minutes. The mean proportion of total sleep time spent in each sleep stage was $6.11 \pm 2.77\%$ for N1, $51.31 \pm 8.62\%$ for N2, $19.39 \pm 6.29\%$ for N3, and $23.19 \pm 6.59\%$ for REM. These values are in line with previous reports on the duration and architecture of sleep in older individuals (Klerman & Dijk, 2008; Ohayon et al., 2004).

In line with the literature, when focusing on sleep slow waves, we found a main effect of sex on overnight NREM SWE ($F_{1,97} = 8.02, p = .006, R^2_{\beta^*} = .08$), with females generating more sleep slow waves compared to males ($t = -2.83, p = .006$; **Fig. III.4A**). Using Pearson's correlation, we further found a significant age-related decrease in NREM SWE ($r = -.24, p = .01$; **Fig. III.4B**), which was reduced to a statistical trend when adjusting for the effect of sex in the GLMM ($F_{1,97} = 2.95, p = .09$).

Age, slow wave energy, and brain (micro-)structure

Pearson's correlations revealed significant and positive links between NREM SWE and quantitative parameters of MT ($r = .25, p = .01$), PD ($r = .29, p = .003$), and R1 ($r = .21, p = .04$; **Fig. III.5A-C**). Strikingly, all these associations were positive, *i.e.* opposite to the effect of age. By contrast, neither GM density ($r = .05, p = .62$) nor R2* ($r = .12, p = .23$) were significantly associated with NREM SWE (**Fig. III.5D-E**).

In a final set of GLMMs, we aimed to confirm the highlighted relationships while adjusting for the effect of age, sex, education, and total sleep duration (**Table III.2**). Similar associations as those observed with simple correlations were found between NREM SWE and

MT ($F_{1,95} = 4.23$, $p = .04$, $R^2_{\beta^*} = .04$) or PD ($F_{1,95} = 7.67$, $p = .007$, $R^2_{\beta^*} = .08$). By contrast, R1 parameter was no longer significantly associated with NREM SWE ($F_{1,95} = 2.17$, $p = .14$). The effect of R2* and GM density remained non-significant (both $p > .05$).

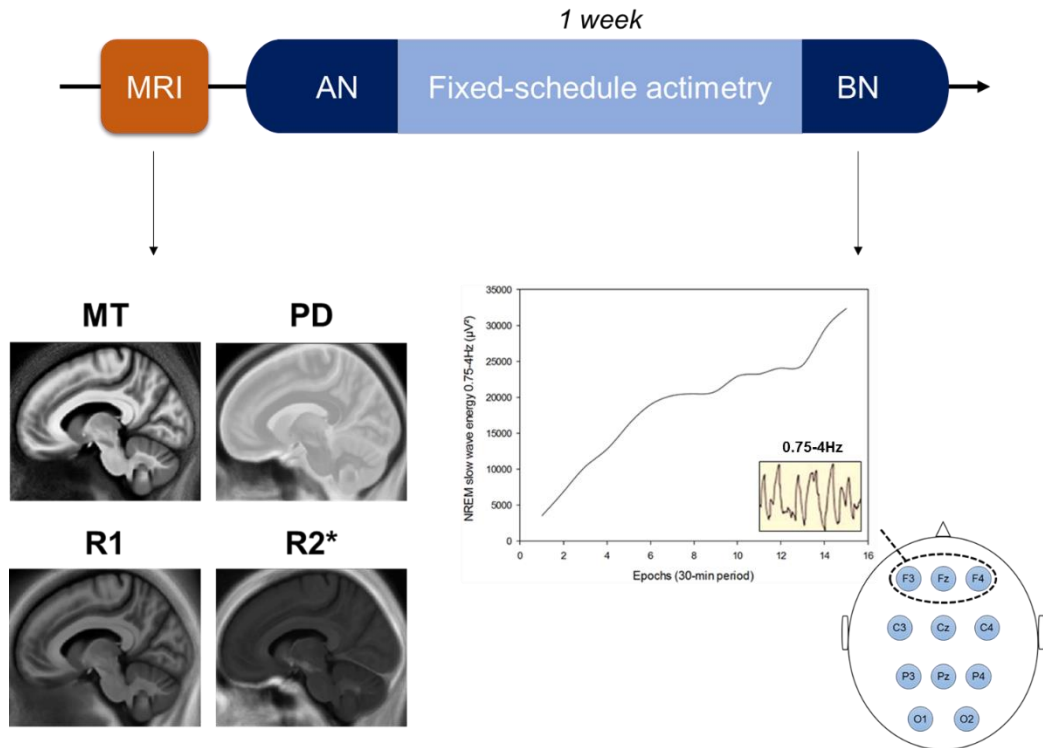


Figure III.1. Overview of the experimental design. Quantitative maps related to magnetization transfer (MT), proton density (PD), as well as longitudinal (R1) and effective transverse (R2*) relaxation rates were acquired using a multi-parametric imaging protocol. After an adaptation night and one week of fixed sleep-wake schedule verified by actimetry, participants' habitual sleep was recorded under EEG and NREM slow wave energy (SWE) was investigated over the frontal derivations (see methods). MRI: magnetic resonance imaging; AN: adaptation night with polysomnography to screen for sleep apnea; BN: baseline night. Quantitative maps used for illustration correspond to the averaged whole-brain maps across the sample.

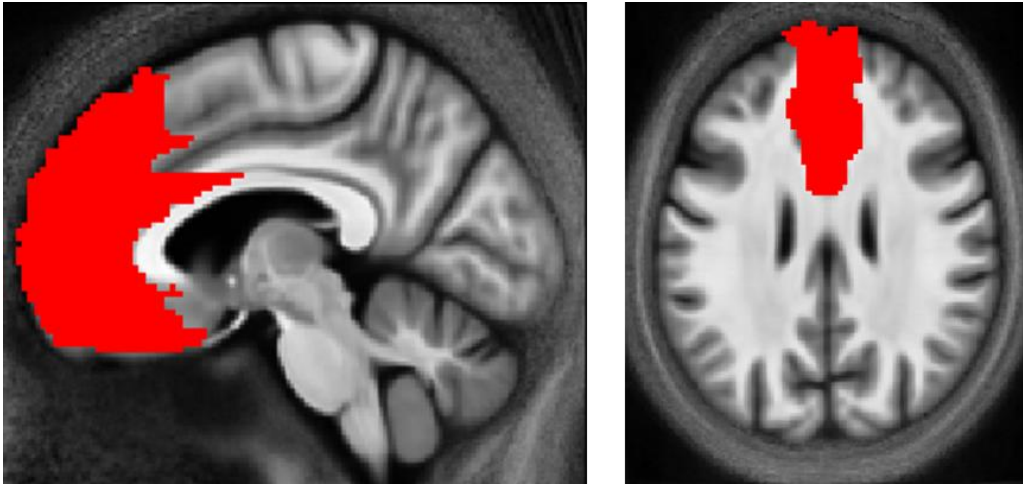


Figure III.2. Visualization of the composite medial prefrontal region of interest. Based on the Automated Anatomical Labeling atlas (Tzourio-Mazoyer et al., 2002), a composite mask was built which included bilateral medial prefrontal, medial orbital, medial orbito-frontal, rectus, and anterior cingulate cortices. For each quantitative MRI parameters, the mean value was extracted within this region. The mask is displayed on the average MT map across subjects.

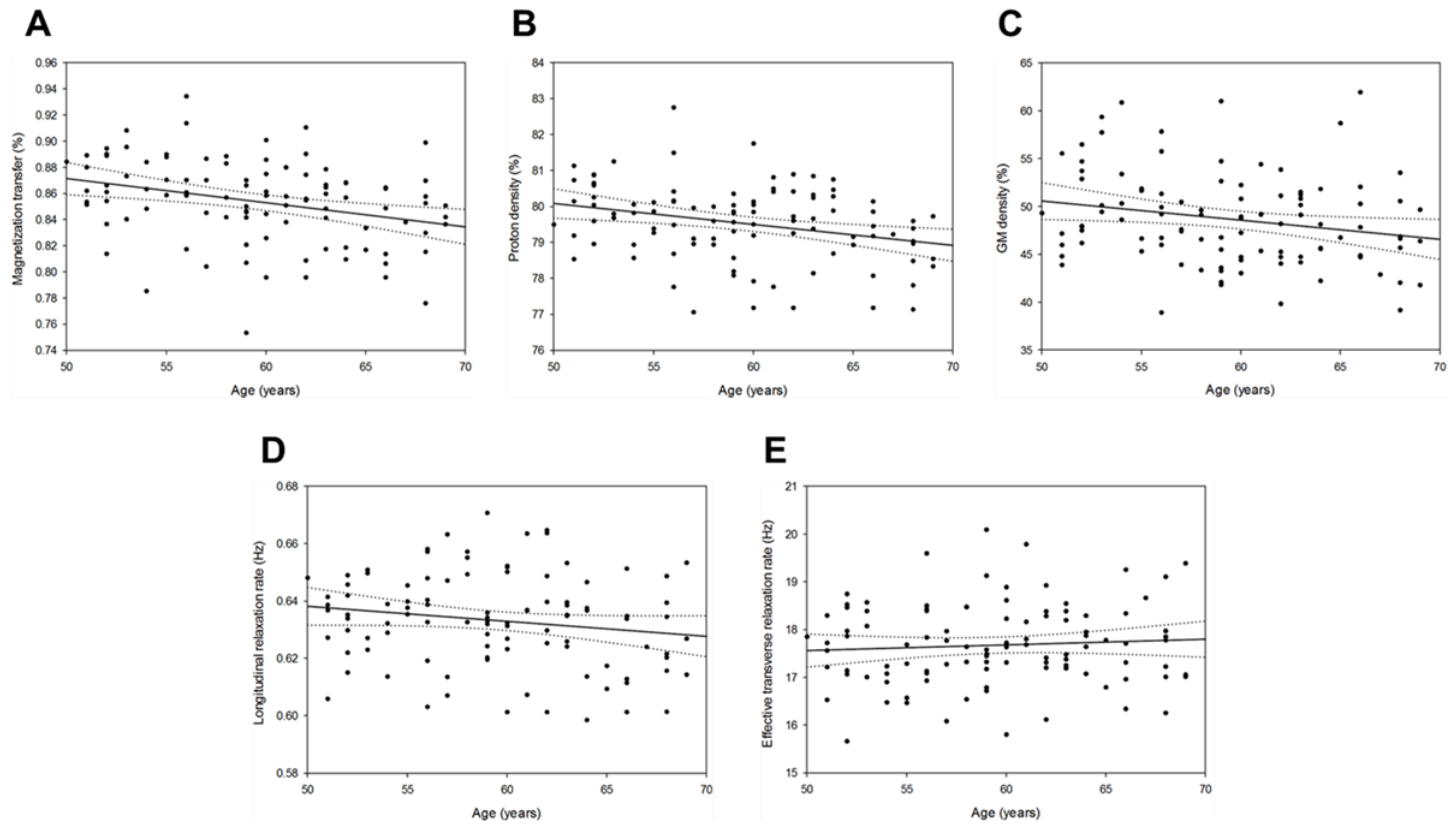


Figure III.3. Relationships between age and mean quantitative parameters in the medial prefrontal cortex. **A)** Significant and negative association between age and magnetization transfer saturation index ($F_{1,97} = 10.56$, $p = .002$, $R^2_{\beta^*} = .10$). **B)** Significant and negative association between age and proton density index ($F_{1,97} = 9.46$, $p = .003$, $R^2_{\beta^*} = .09$). **C)** Significant and negative association between age and grey matter density ($F_{1,97} = 7.51$, $p = .008$, $R^2_{\beta^*} = .07$). **D)** No significant association between age and longitudinal relaxation rate ($F_{1,97} = 2.84$, $p = .10$). **E)** No significant association between age and effective transverse relaxation rate ($F_{1,97} = 0.47$, $p = .49$).

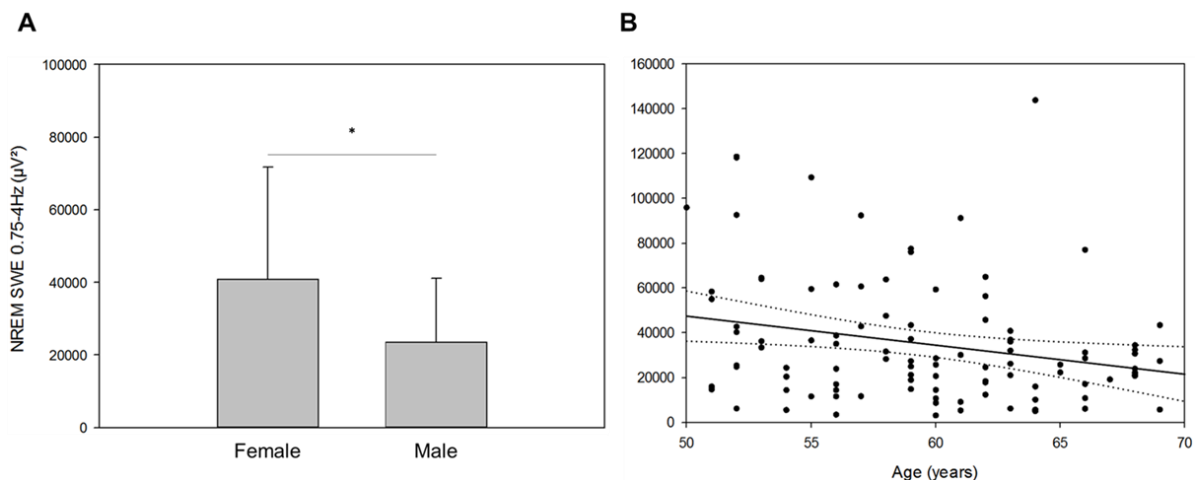


Figure III.4. Sex- and age-related decrease in NREM sleep slow waves generation. **A)** When compared to males, females displayed an increased amount of NREM SWE ($t_{1,97} = -2.83, p = .006$). **B)** Age was significantly associated with reduced NREM SWE (Pearson's correlation: $r = -.24, p = .01$), but this relationship became a statistical trend when accounting for the effect of sex (GLMM: $F_{1,97} = 2.95, p = .09$). * $p < .01$.

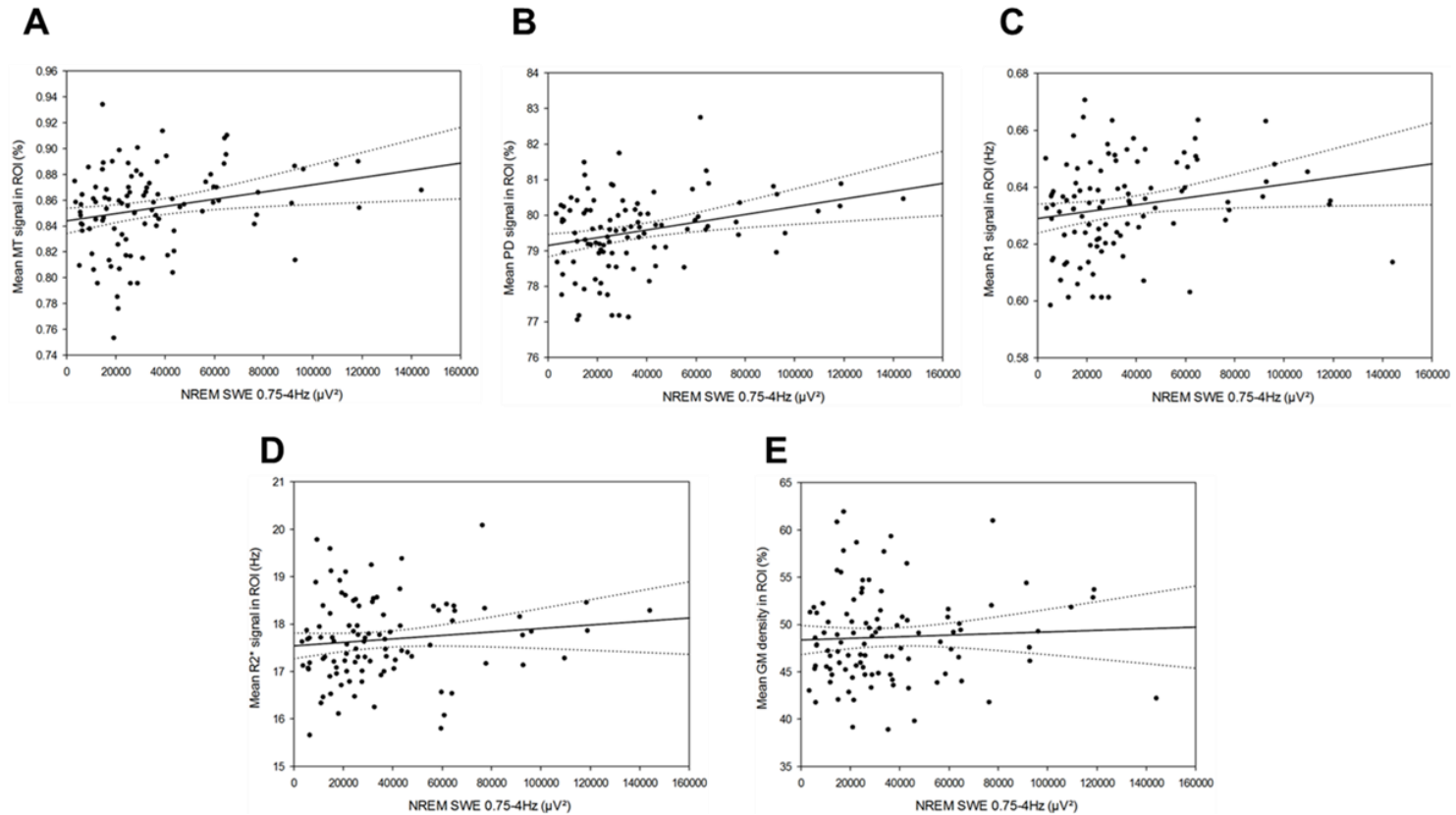


Figure III.5. Relationships between NREM SWE and quantitative parameters in the medial prefrontal cortex. **A)** Significant and positive association between NREM SWE and mean MT signal ($r = .25, p = .01$). **B)** Significant and positive association between NREM SWE and mean PD signal ($r = .29, p = .003$). **C)** Significant and positive association between NREM SWE and mean R1 signal ($r = .21, p = .04$). **D)** No significant association was found between NREM SWE and mean R2* signal ($r = .12, p = .23$). **E)** No significant association was found between NREM SWE and mean GM density ($r = .05, p = .62$).

Table III.1. Demographic characteristics of the study sample (mean $\pm$ SD).

	N = 101
Sex	68F/33M
Age (years)	59.44 $\pm$ 5.29
Education (years)	15.16 $\pm$ 3.01
Ethnicity	Caucasian
Baseline night total sleep duration (min, EEG)	391.81 $\pm$ 45.38
Time spent in sleep stage N1 (% of total sleep duration, EEG)	6.11 $\pm$ 2.77
Time spent in sleep stage N2 (% of total sleep duration, EEG)	51.31 $\pm$ 8.62
Time spent in sleep stage N3 (% of total sleep duration, EEG)	19.39 $\pm$ 6.29
Time spent in sleep stage REM (% of total sleep duration, EEG)	23.19 $\pm$ 6.59
Mean magnetization transfer saturation (% ROI)	0.85 $\pm$ 0.03
Mean proton density (% ROI)	79.54 $\pm$ 1.05
Mean longitudinal relaxation rate (Hz, ROI)	0.63 $\pm$ 0.02
Mean effective transverse relaxation rate (Hz, ROI)	17.68 $\pm$ 0.86
Mean grey matter density (% ROI)	48.70 $\pm$ 4.84

F = female; M = male; EEG = electroencephalography; ROI = region of interest.

Table III.2. GLMM outputs of the associations between quantitative multiparametric parameters in the ROI and NREM SWE, after adjusting for covariates of age, sex, education, and total sleep time.

		Dependent variables				
		MT	PD	R1	R2*	GM density
Regressors	Age	F_{1,95} = 8.26	F_{1,95} = 6.95	F _{1,95} = 1.43	F _{1,95} = 0.91	F_{1,95} = 5.74
		p = .005	p = .01	p = .23	p = .34	p = .02
		R²_{β*} = 0.08	R²_{β*} = 0.07			R²_{β*} = 0.06
	Sex	F _{1,95} = 0.36	F _{1,95} = 1.00	F _{1,95} = 0.01	F _{1,95} = 0.58	F_{1,95} = 13.22
		p = .55	p = .32	p = .99	p = .45	p < .001
						R²_{β*} = 0.12
	Education	F _{1,95} = 1.11	F _{1,95} = 0.04	F _{1,95} = 2.80	F _{1,95} = 0.27	F _{1,95} = 2.41
		p = .30	p = .84	p = .10	p = .60	p = .12
	Total sleep time	F _{1,95} = 1.09	F _{1,95} = 1.74	F _{1,95} = 1.33	F _{1,95} = 0.57	F _{1,95} = 0.08
		p = .30	p = .19	p = .25	p = .45	p = .77
	NREM SWE	F_{1,95} = 4.23	F_{1,95} = 7.67	F _{1,95} = 2.17	F _{1,95} = 2.86	F _{1,95} = 1.02
		p = .04	p = .007	p = .14	p = .09	p = .31
R²_{β*} = 0.04		R²_{β*} = 0.08				

*R*²_{β*} values correspond to semi-partial *R*² in GLMMs, and are reported for all significant associations.

Discussion

Physiological aging is inherently accompanied by modifications in brain structure that are associated with a disruption of sleep and wakefulness regulation, most likely in a bidirectional manner (Musiek & Holtzman, 2016; Van Egroo, Narbutas, Chylinski, Villar González, Maquet, Salmon, Bastin, et al., 2019). Previous studies used macrostructural measures of brain structural integrity to investigate the brain bases of the decrease in NREM sleep slow waves generation in aging (Saletin et al., 2013) (Dubé et al., 2015; Mander et al., 2013). Here, we tested whether tissue microstructural properties could also underlie this age-related decline, using a state-of-the-art quantitative multiparametric MRI protocol. We found that a reduction in the amount of overnight NREM SWE was associated with a reduction in magnetization transfer saturation and proton density in a set of medial prefrontal areas as well as with longitudinal relaxation rates when not accounting for the effects of age and sex.

MT signal reflects macromolecular content and shows a strong specificity to myelin, as established by concurrent histological measures of *ex vivo* tissues (Schmierer, Scaravilli, Altmann, Barker, & Miller, 2004; Schmierer et al., 2007). Recently, a quantitative multiparametric MRI study in a sample 138 healthy individuals aged 19-75 years showed that the rate of the age-dependent decrease in MT signal over the whole-brain was ranging from 0.01% to 0.03% per decade of life, and was around 0.025% in the medial frontal cortex (Callaghan et al., 2014). In line with our hypothesis, we further found a positive association between MT signal and NREM SWE, which was therefore in opposite direction compared to the association with age. Our results therefore suggest that preserved myelination in the medial frontal grey matter may help maintaining sleep slow waves generation in older individuals. Increased myelination may facilitate neuronal synchronization through enhanced/more efficient connections between neuronal populations, resulting in higher power density in the slower frequency band. This straightforward rationale is supported by evidence linking myelin, neuronal synchronization, and slow oscillations in animal and computational models (Pajevic, Basser, & Fields, 2014; Vyazovskiy et al., 2009).

The interpretation of our findings related to PD is not as clear. Increase in PD signal has for instance been linked to brain lesions in multiple sclerosis (Gracien et al., 2016; Stüber et al., 2014). However, in non-pathological aging, a higher PD signal may mostly reflect a higher neuronal content. Our data support this view, as we find a positive association between grey matter volume and proton density, although not reaching statistical significance

(Pearson's correlation: $r = .18$; $p = .07$). The association we find between SWE and PD signal in the medial prefrontal cortex may thus arise from a higher neuronal content which would contribute to increased EEG power. Opposite to this assumption and contrary to some previous studies (Buchmann, Ringli, et al., 2011; Mander et al., 2013; Saletin et al., 2013) – and yet in line with others (Buchmann, Kurth, et al., 2011) – we found no significant association with medial prefrontal GM volume, which was not the focus of the present paper. Cortical thickness may constitute a more appropriate mean to isolate relationships between brain structure and EEG measures (Buchmann, Kurth, et al., 2011), as in previous paper linking reduced cortical thickness to sleep slow waves in aging (Dubé et al., 2015).

Longitudinal relaxation rate correlates not only with myelin, but also with iron concentration (Stüber et al., 2014). Since its association with SWE was not significant when correcting for age and sex, we will not comment on the potential association that we observed in simple correlation analysis. In addition, we found no relationship between effective transverse relaxation rate and NREM SWE. This was expected as $R2^*$ signal is especially sensitive to iron deposits, which have been mostly observed in subcortical nuclei or in early-myelinating cortical regions outside the medial prefrontal cortex in older individuals. In addition, abnormal brain iron accumulation has been reported in sleep disorders such as primary insomnia (Lin Chen, Wei, Liu, Li, & Zhou, 2019) but our participants have been thoroughly screened to ensure that they were devoid of such conditions.

Our results rely on a relatively large sample so that our negative findings are unlikely to arise from an insufficient statistical power. Brain microstructure inferences were based on state-of-the-art data acquisition and processing approaches (Tabelow et al., 2019). Yet, several limitations to our protocol may be considered: first, whereas the literature strongly supports the implication of the frontal regions in aging, both with regard to brain structural integrity and sleep slow waves, the regional aspect of the relationships highlighted here should be extended to the whole-brain. In addition, while we quantified the cumulative amount of power density in the delta band (0.75-4Hz), future studies should assess the links between quantitative multiparametric parameters and more specific characteristics of these slow oscillations, such as the slope, amplitude, or duration among the hyperpolarized and depolarized phases, which inform about different cellular mechanisms involved in cortical neurons synchronization (Amzica & Steriade, 1998; Carrier et al., 2011; Neske, 2016). Finally, the advantages of reaching *in vivo* histology through quantitative multiparametric MRI would exponentially benefit from ultra-high field neuroimaging techniques (Nagel et al.,

2018), which become increasingly available worldwide. As deep pyramidal cells provide most of the recorded EEG signal at the electrode level (Cohen, 2017), characterizing quantitative MRI parameters within cortical layers would refine our understanding of the complex link between synchronized activity and structure among neuronal populations.

An increasing body of evidence show that the quality of sleep and wakefulness regulation is of prime importance to sustain cognition in normal and pathological aging (Gagnon et al., 2019). Quantification of NREM sleep slow waves has been associated with higher amyloid-beta ($A\beta$) burden in the medial prefrontal cortex (Mander et al., 2015), while experimental reduction of sleep slow waves disturb $A\beta$ content in the CSF (Ju et al., 2017). It is therefore tempting to interpret, based on these evidence, that preserved myelin content might protect against neurodegeneration through its positive impact on sleep slow waves, and ultimately constitute a positive factor in successful cognitive aging. A first step to test this speculation would be to assess whether myelin content is associated with regional $A\beta$ and whether it could be linked to cognition in older individuals.

In summary, our cross-sectional study shows that brain microstructural aspects related to myelin content, as measured in the medial prefrontal lobe, are associated with the preserved ability to generate slow waves during NREM sleep in healthy older adults. These findings may have implication for successful aging and neurodegenerative diseases.

General discussion

Despite unceasing technological progress and medical discoveries, one cannot escape the pervasive effects of brain physiological aging. With increasing age comes a series of inevitable alterations in brain structure and function that interact with genetic and lifespan factors to result in a wide range of possible cognitive trajectories from one individual to another (Papenberg, Lindenberger, & Bäckman, 2015; Reuter-Lorenz & Park, 2014; Scheltens et al., 2016). In order to counter the increasing prevalence of dementia, which is inherent to our ever-aging societies, research has emphasized the benefits of preventing in opposition to treating; that is, by identifying modifiable factors that can delay the broad impact of aging on cognition, such as mentally stimulating activities, social activity, exercise, or nutritional habits (Daffner, 2010; Y. Wang, Du, Li, & Qiu, 2019). In addition to these factors, age-related changes in the regulation of sleep and wakefulness have been increasingly recognized as crucial in brain maintenance and in protecting against cognitive decline, especially with regard to AD (Leng, Musiek, Hu, Cappuccio, & Yaffe, 2019; Yaffe et al., 2014). Given that efficient treatments for AD remain elusive, delaying the onset of symptoms by working on the aforementioned modifiable factors, including sleep-wake regulation, may constitute the most realistic alternative approach and is thought to hold promising socio-economic outcomes (Zissimopoulos et al., 2015).

In the quest for so-called ‘successful cognitive aging’ (Daffner, 2010), we aimed to show that the temporal organization of neuronal function during wakefulness and sleep may constitute an important marker in the intricate relationships that connect brain structure, brain function, and sleep-wake regulation in older individuals. In this final section, we will further discuss the results presented in this thesis and we will introduce several research perspectives that may arise from our findings, while accounting for the strengths and limits of our research protocol.

Confronting theoretical models

In light of the synaptic homeostasis hypothesis (SHY), the wake-dependent changes of cortical excitability, as measured by the slope of the TMS-evoked EEG potential, is thought to reflect the homeostatic increase in synaptic strength (Kuhn et al., 2016). However, our empirical observations that a major part of the sample used in Chapter I displayed a progressive decrease in cortical excitability values during prolonged wakefulness do not seem

to be in line with SHY predictions. With regard to the local use-dependent modulation, participants are mostly administered executive tasks throughout the protocol (*i.e.* 3 out of the 4 tasks included in the test battery repeated 9 times) and cortical excitability is measured over the frontal cortex. SHY would therefore predict that synaptic strengthening, as reflected by an overall increase in cortical excitability values, should be particularly evident in the frontal region. Again, this is supported by only a minor proportion of our sample of 60 individuals. Similarly, SHY postulates that theta activity during wakefulness is another marker of net synaptic strength (Finelli, Baumann, Borbély, & Achermann, 2000; Tononi & Cirelli, 2006). Consequently, the temporal dynamics of theta activity as measured by resting-state wakefulness-EEG recordings should both display an overall increase and follow cortical excitability dynamics, which as we demonstrated, is quite incompatible. Extending our analysis to the final sample of 101 participants and exploring other parameters related to sleep homeostasis and synaptic strengthening should contribute to determine how our data fit with predictions from SHY. To better answer that question, future studies based on our findings should also consider the addition of an EEG recording of the recovery night following wakefulness extension, as well as a supplementary TMS-EEG session after the recovery night. These data would better inform about how sleep helps recalibrate net synaptic strength. Going in that direction, it may be possible that such sleep-dependent synaptic re-normalization is promoted by increased brain microstructural integrity, such as the preserved grey matter myelination observed in association with increased NREM sleep slow waves in Chapter III. However, the lack of clear mechanistic pathways provided by SHY remains a significant issue in further characterizing the role played by the relationships that we highlighted in synaptic rescaling. In addition, SHY does not account for circadian influences on wake-dependent changes in brain function, which in our particular case have been established to modulate cortical excitability (Ly et al., 2016), in an age-dependent manner (Gaggioni et al., 2019). This circadian regulation of cortical excitability may correlate with changes in body temperature dynamics or in melatonin secretion profiles, which are among the well-established circadian signals affected by physiological aging (Wu & Swaab, 2005, 2007). Supplemental analyses not presented in Chapter I found no significant associations between cortical excitability regulation profiles and these circadian parameters, but it is important to remind that our protocol is not optimized to examine circadian aspects.

Overall, the results related to tau protein burden in Chapter II are in line with the landmark observations of tau pathology progression (Braak & Del Tredici, 2011; Braak, Thal,

Ghebremedhin, & Tredici, 2011; Vogels et al., 2019), although we cannot formally assess whether tau protein burden was first present in the brainstem or in the medial temporal lobe in our cross-sectional study. Interestingly, the co-presence of tau protein in certain cortical areas such as in the insula or fusiform gyrus would need to be replicated and further investigated, especially with more specific radioligands, as these areas are usually affected in the middle stages of tau pathology progression (Maass et al., 2017). Nevertheless, we were able to provide a set of associations that support the specific role of the early tau aggregates in the brainstem for cortical function.

Finally, although requiring further validation, the relationships presented in the last results section may be interpreted in the context of cellular models of sleep slow waves. Essentially, slow waves consist of recurrent transitions from periods of synchronized neuronal activity ('ON state') to periods of synchronized silence ('OFF state') among neuronal populations (Vyazovskiy, Riedner, Cirelli, & Tononi, 2007), which is reflected at the scalp level by an overall slower EEG activity. Our results suggest that this characteristic of synchronization, crucial for sleep slow waves, may be promoted by a higher tissue myelin content, potentially through a facilitating cross-talk between local and distant neuronal populations which eventually results in higher power in the slower frequency band at the scalp level. Exploring the associations between regional myelin and A β or tau contents should provide further understanding of the biophysical foundations underlying the observed relationships, as the release of A β and tau proteins has been tightly linked to neuronal activity (Cirrito et al., 2005; Kang et al., 2009; Yamada et al., 2014).

In summary, our findings are partially in line with theoretical models linking sleep-wake regulation, brain structure, and brain function, but they also raise intriguing new research questions. In the following sections, we will discuss considerations that must be carefully examined to decipher our results.

Specificity of the results

A first consideration in the interpretation of our findings relates to the specificity of the study sample. All results are based on a group of older individuals who were devoid of many of the well-established risk factors associated with cognitive decline and AD (Baumgart et al., 2015). As illustrated by the relative difficulties to recruit participants who meet the extensive list of inclusion criteria, one could therefore question the value of the highlighted associations for the general population. In a utopic scientific context, it may be desirable to

control most, if not all, factors that could influence the observed associations between brain structure, brain function, and sleep-wake regulation. Yet, doing so may actually impede the process of disentangling data complexity, as illustrated by the following example: it was recently showed that sleep physiology was associated with gut microbiome diversity, which in turn has been linked to immune system biomarkers (Smith et al., 2019). The latter is considered as an additional important factor in the pathogenesis of AD (Talwar, Kushwaha, Gupta, & Agarwal, 2019) and displays a circadian regulation (Man, Loudon, & Chawla, 2016). In addition, gut microbiome diversity has been linked to oral bacteria (Olsen & Yamazaki, 2019) which, surprisingly, have recently been considered as a potential key factor in AD pathophysiological processes (Dominy et al., 2019), that are in turn thought to affect sleep physiology and circadian rhythmicity (Dufort-Gervais et al., 2018; Ju et al., 2014; C. Wang & Holtzman, 2019). Without a doubt, this chain of identified associations is an inherent benefit of the rise of multidisciplinary scientific methods and contributes to the holistic approach to the unknown, but its disadvantages may reside in an over-complexification of mechanistic models and in potential circular pitfalls. An efficient way to parsimoniously define the most relevant confounding factors to consider when designing a future study may be to perform a set of meta-analyses of empirical data in order to extract the factors that have been proved to show the strongest associations with the variables of interest. One could also build a composite index based on these factors and include it in statistical models to ensure that the potential new associations overcome the variance explained by these confounding variables. Regarding the risk of declaring AD, a similar concept has been introduced through a frailty index, which aims to account for comorbidities, signs, and symptoms (Wallace et al., 2019).

A second consideration related to the characteristics of the study sample and inherent to the cross-sectional design is the specificity of the highlighted associations across the lifespan. For example, the positive relationship between cognitive performance and preserved cortical excitability regulation profile presented in Chapter I may be stable from early to late adulthood. In other words, if we had tested the same individuals in their younger age, they might have produced similar scatter plots with differences only observed in absolute values. This is of course speculative, as inter-individual lifespan characteristics may also modulate how these relationships evolve with increasing age. Yet, analysis of existing data among younger individuals from a separate study in almost identical experimental settings (Gaggioni et al., 2019) could help disentangle the temporal specificity of the relationships that we found,

at least for Chapter I and III. Another approach would have to be used to answer that question for Chapter II, as the early soluble form of hyperphosphorylated tau protein is not quantifiable *in vivo* with modern techniques.

Third, it remains to be established whether our results may depend on the nature of the variables that we decided to include in our analyses. On the one hand, although we have a strong rationale to focus on the frontal regions, built on multiple previous studies using similar methods, it would be of great interest to further characterize the regional modulation that could be intrinsic to our findings. We may address it either by TMS-EEG assessments over multiple cortical areas for Chapter I and II or by following a topographical approach of sleep slow waves and microstructure across the whole-brain for Chapter III. On the other hand, exploring additional available variables, such as the wake-dependent dynamics of theta activity based on wakefulness-EEG measures (Cajochen, Brunner, Krauchi, Graw, & Wirz-Justice, 1995) or other brain microstructural indices based on neurite orientation density and dispersion imaging (NODDI) (H. Zhang, Schneider, Wheeler-Kingshott, & Alexander, 2012), may reveal broader, less specific associations between our measures. Not shown in the present thesis, we explored on a subset of 70 individuals from our sample whether neurite density, as measured with NODDI-oriented processing and modeling scheme of DWI data, could be related to our sleep-derived measure of homeostatic regulation in addition to the other quantitative parameters that we assessed. In that exploratory analysis, we were not able to isolate significant relationships between neurite density and NREM SWE, suggesting that it may not play a straightforward role in sleep-wake regulation in aging. Nonetheless, other methods may provide more positive results, as NODDI is a quite recent technique requiring further validation and optimization (Tariq, Schneider, Alexander, Gandini Wheeler-Kingshott, & Zhang, 2016).

Notwithstanding these concerns related to specificity, being able to identify significant relationships with small-to-moderate effect sizes in our data (mostly around $R^2_{\beta^*} = 0.10$; ranging from $R^2_{\beta^*} = 0.05$ to $R^2_{\beta^*} = 0.23$) is encouraging, considering that the study sample has a restricted range of inter-individual variability. If replicated, our findings may provide a significant addition to the complex triangular scheme between sleep-wake regulation, brain structure, and brain function in human aging. In addition, they may constitute a solid ground to guide future studies towards elucidating the directionality of these relationships.

Predictive value of the results

While the central role of fundamental research is to acquire extensive knowledge of complex phenomena, one of its ultimate benefits is also to reach practical applications based on empirical observations; in the field of neuroscience, this means feasible clinical translation of the highlighted experimental associations. Overall, our findings contribute to the identification of new markers that may be considered in the diagnosis and prognosis of older individuals questioning their cognitive status. The longitudinal assessment of cognitive functions after 2 years, which is still ongoing for a majority of our participants, will bring crucial hints towards the predictive aspect of our work. Based on the present results, we may hypothesize that, in our sample, older individuals with young-like regulation profiles of cortical excitability will display a less marked cognitive decline after 2 years. The importance of our markers of brain structural integrity may, this time, appear as potential mediating factors in individual cognitive trajectories. Similarly, individuals with preserved brain structural integrity, whether it be regarding early tau protein aggregates or microstructural quantitative parameters, may be more protected against cognitive alterations. The latter assumption is highly speculative as we did not thoroughly assess the relationships between these variables of brain integrity and cognition in analyses from Chapter II and III.

The major frontal involvement in all our findings further supports the well-described anterior-posterior discrepancies in sleep-wake regulation as well as in brain structure and function in the course of aging (S. W. Davis, Dennis, Daselaar, Fleck, & Cabeza, 2008; Marzano, Ferrara, Curcio, & De Gennaro, 2010; Nyberg et al., 2010; Schmidt et al., 2012). Thus, probing function and integrity of the frontal cortex may be especially useful in clinical practice as this region seems particularly prone to the effects of physiological aging. In addition to that, Chapter II suggests that granting particular attention to alterations in the brainstem, and potentially the LC, may inform about the integrity of cortical function, which is in line with the increasing interest devoted to this subcortical structure in the study of cognitive aging (Dahl et al., 2019; Jacobs, 2019).

One could hardly imagine, however, that patients would undergo a wakefulness extension protocol of 20 h in constant routine conditions in order to characterize their cortical excitability regulation profile. Therefore, the associations presented in Chapter I still need to be further explored in order to identify correlates that could be assessed in a clinical context in order to envision realistic translation of our results. For example, if we were to show that

these wake-dependent modulations of cortical functions are reflected at the individual level in the temporal organization of the rest-activity cycle measured with actigraphy, an easy-to-use and non-invasive device, then it would provide clinicians with new tools to improve therapeutic management of patients.

Additional perspectives

Beyond the results presented here, the COFITAGE study holds many more promises. Thanks to the relentless work of my dearest PhD fellows, we will soon be in position to further disentangle the complicated relationships between sleep-wake regulation, brain structure, and brain function in healthy aging. Ongoing analyses include investigations of how A β and tau protein burden may be associated with microstructural aspects of sleep fragmentation (*e.g.* arousals) or with reserve factors (*e.g.* allostatic load, cognitive reserve), and the associated contributions of all these factors to cognition.

Our next focus will deal with cortical excitability dynamics and the time course of cognitive performance within the prolonged wakefulness protocol, based on the nine repeated neuropsychological assessments. Whereas Chapter I presented a link between the wake-dependent dynamics of cortical excitability and traits of cognition, we still have to investigate whether and how cognitive performance during a mild wakefulness extension challenge is modulated by variations in cortical excitability. This was previously investigated in a smaller sample in our lab and yielded encouraging results (Gaggioni et al., 2019), but the multi-modal aspect of the COFITAGE study and our larger sample allow for a much more comprehensive approach, while accounting for potential confounding factors related to brain structural integrity not available in previous studies.

As mentioned before, mechanistic processes underlying the wake-dependent variations in cortical excitability may be identified with an *in silico* approach such as dynamic causal modeling of TMS-evoked potentials using neural mass models (Friston, Harrison, & Penny, 2003). It was previously shown that temporal variations in measures of excitatory/inhibitory connectivity and in GABA/glutamate balance were closely related to cortical excitability dynamics (Chellappa et al., 2016). Therefore, we also aim at extending these results to our sample of older individuals, as it remains unknown how aging may affect mesoscopic factors underlying cortical excitability.

Finally, we will investigate the local relationships between the different markers of brain structural integrity available through the multiple neuroimaging modalities in our data. We will require the use of a robust biological parametric mapping approach, which allows voxel-wise assessments of quantitative parameters across multiple imaging modalities (X. Yang, Beason-Held, Resnick, & Landman, 2011). This may provide critical information about how, for example, focal measures of myelin content and A β or tau protein burden are correlated, for each voxel over the whole brain.

Conclusions

It is truly fascinating that, although sleep has been thoroughly investigated for decades, new mechanisms related to its multiple functions are still being discovered. Yet, despite being so central to normal functioning, the relationships between sleep-wake regulation, brain structure, and cognition still entail many mysteries. The present work is inscribed in the recent research efforts to account for these triangular connections in the course of aging. By using a multi-modal approach of these concepts, we showed that wake-dependent regulation of cortical function is tightly associated with cognition in healthy older individuals. In addition, we demonstrated that the baseline level of cortical excitability during wakefulness is specifically linked to early AD-related alteration in the brainstem. Finally, we showed that sleep-derived measures of cortical function are associated with markers of brain microstructural integrity. Our findings are partially in line with the literature, but also raise new intriguing research questions. While several pieces of the global picture remain to be unveiled, the numerous data yielded by our protocol hold many more promises for the future, especially regarding the specificity of our findings and their predictive value.

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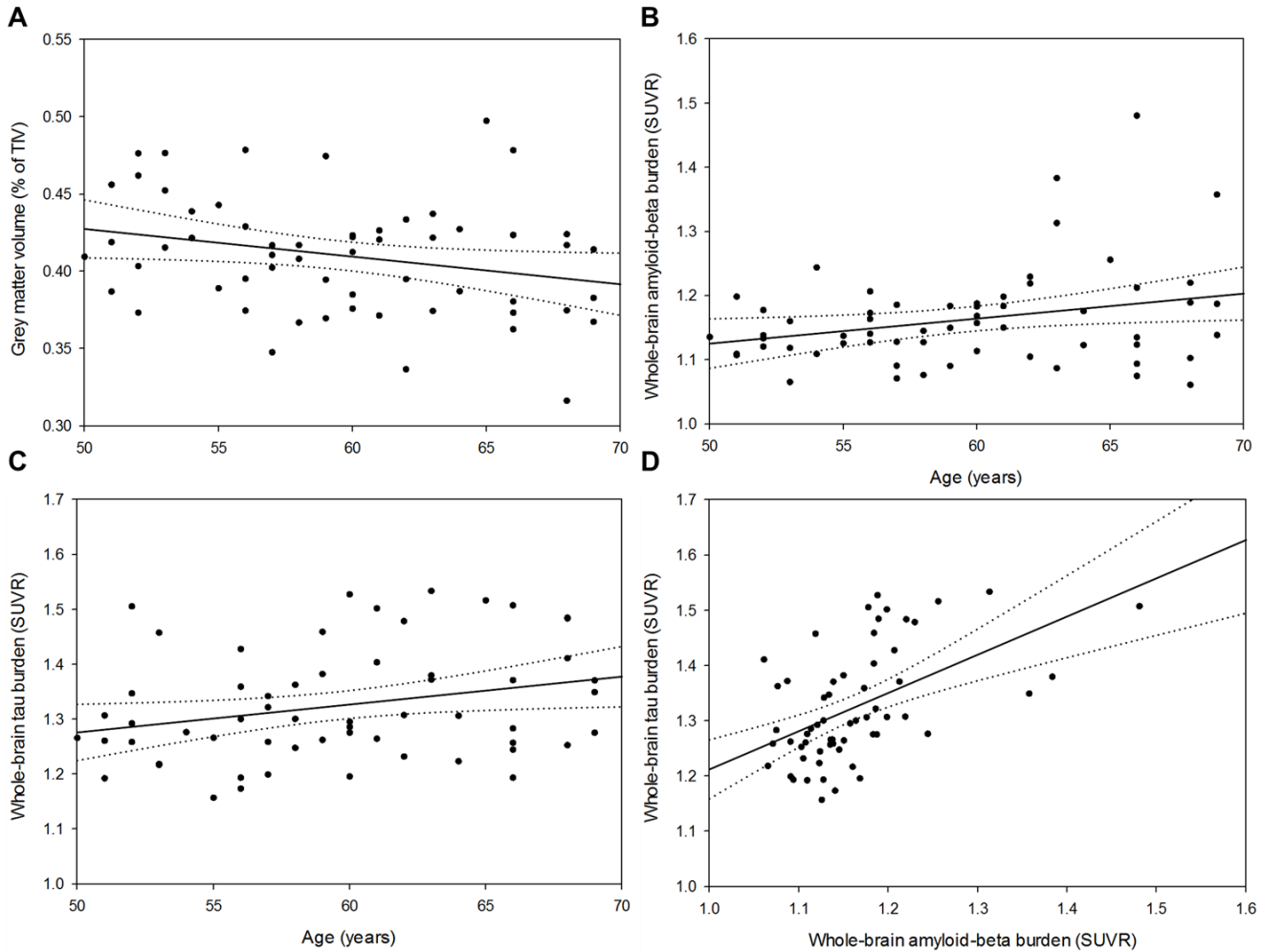
List of publications

- Van Egroo, M.**, Narbutas, J., Chylinski, D., Villar González, P., Maquet, P., Salmon, E., ... Vandewalle, G. (2019). Sleep-wake regulation and the hallmarks of the pathogenesis of Alzheimer's disease. *Sleep*, 42, zsz017.
- Van Egroo*, M.**, Narbutas*, J., Chylinski*, D., Villar González, P., Ghaemmaghami, P., Muto, V., ... Vandewalle, G. (in press). Preserved wake-dependent cortical excitability dynamics predict cognitive fitness beyond age-related brain alterations. *Communications Biology*.
- Van Egroo, M.**, Chylinski, D., Narbutas, J., Besson, G., Muto, V., Schmidt, C., ... Vandewalle, G. (submitted). Brainstem tau protein burden is associated with cortical hyper-excitability in healthy aging. *The Journal Of Neuroscience*.
- Van Egroo*, M.**, Chylinski*, D., Narbutas, J., Vandeleene, N., Grignard, M., Deantoni, M., ... Vandewalle, G. (in preparation). Higher prefrontal cortex myelination is associated with more sleep slow waves in healthy aging.
- Van Egroo*, M.**, Gaggioni*, G., Cespedez-Ortiz*, C., Ly, J. Q. M., & Vandewalle, G. (2019). Steady-State Pupil Size Varies with Circadian Phase and Sleep Homeostasis in Healthy Young Men. *Clocks & Sleep*, 1(2), 240-258.
- Narbutas*, J., **Van Egroo*, M.**, Chylinski*, D., Villar González, P., Garcia Jimenez, G., Besson, G., ... Collette, F. (2019). Cognitive efficiency in late midlife is linked to lifestyle characteristics and allostatic load. *Aging*, 11(17), 7169-7186.
- Gaggioni, G., Ly, J. Q. M., Muto, V., Chellappa, S. L., Jaspar, M., Meyer, C., ... **Van Egroo, M.**, ... Vandewalle, G. (2019). Age-related decrease in cortical excitability circadian variations during sleep loss and its links with cognition. *Neurobiology of Aging*, 78, 52-63.
- Ghaemmaghami, P., Muto, V., Jaspar, M., Meyer, C., Elansary, M., Koshmanova, E., **Van Egroo, M.**, ... Vandewalle, G. (submitted). Alzheimer's disease genetic liability is linked to sleep slow waves and daytime sleepiness in healthy young men. *Nature Communications*.

Appendix

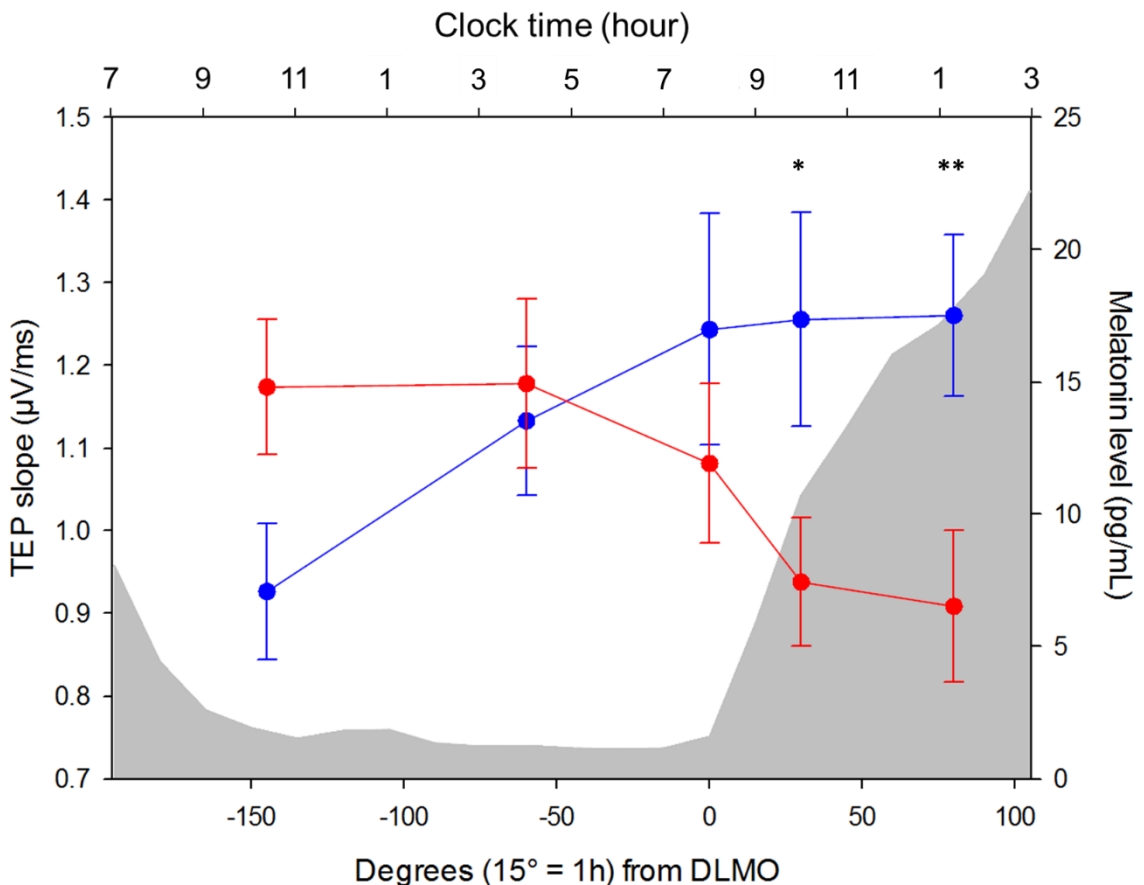
Appendix 1. Supplementary materials for results Chapter I.

Supplementary Figures



Supplementary Figure I.1. Relationships between age and brain integrity measures. **A)** Negative association between age and whole-brain GM volume ($n = 60$; $F_{1,56} = 5.86$, $p = 0.02$, $R^2_{\beta^*} = 0.10$). **B)** Statistical trend towards a positive association between age and whole-brain A β burden ($n = 60$; $F_{1,56} = 3.74$, $p = 0.06$). **C)** Statistical trend towards a positive association between age and whole-brain tau burden ($n = 60$; $F_{1,56} = 3.71$, $p = 0.06$). **D)** Positive association between whole-brain A β protein burden and whole-brain tau protein burden ($n = 60$; $F_{1,55} = 16.00$, $p = 0.0002$, $R^2_{\beta^*} = 0.23$). Simple regressions were used only for a visual display of the direction of the associations. Dotted lines represent 95% confidence interval of these simple regressions. The narrow age range of our relatively young sample

most likely explains that we observe trends rather than significant links between age and A β or tau burdens. Nonetheless, the fact that both PET markers show the expected positive association with increasing age support their validity as markers of brain integrity.



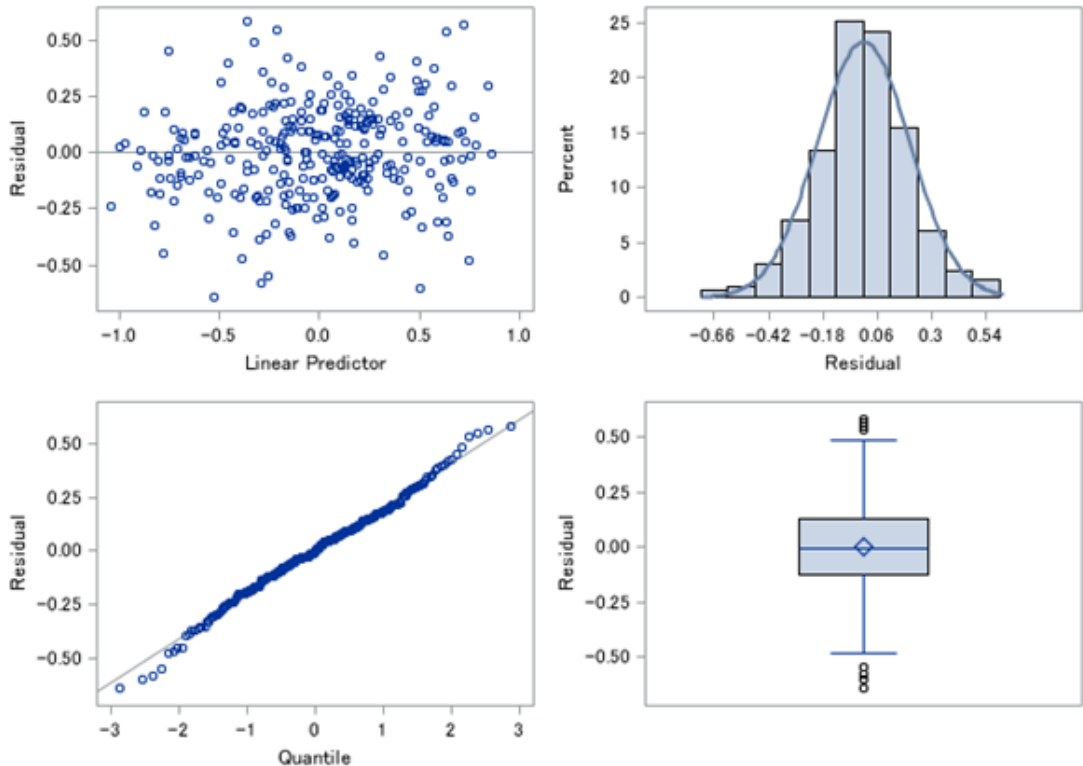
Supplementary Figure I.2. Averaged cortical excitability dynamics of individuals displaying young-like cortical excitability profile (CEP) (blue, $n = 25$) vs. individuals who display old-like CEP (red, $n = 35$). * $p_{adj} < 0.05$; ** $p_{adj} < 0.01$. Importantly, except for analyses related to this figure, CEP was considered as a continuous variable for all statistical analyses in the present study.

Supplementary Methods

Cortical excitability profile index validation

When analysing the time course of cortical excitability values across the 5 phases of TMS-EEG acquisitions, residuals from the linear modeling verified the goodness-of-fit across the sample. Furthermore, we computed the difference between the last and the first TMS-EEG sessions (TEP slope S5-S1), so that the ‘young-like’ increase in cortical excitability during the biological night compared to the biological day would be also reflected in a positive value. The proportions of young-like vs. old-like subjects measured with CEP or TEP slope S5-S1 were identical (*i.e.* 25 young-like vs. 35 old-like in both cases), and the individual attribution to young-like or old-like profiles were highly matched between the two indices (Phi coefficient = 0.86; only 4 individuals were not attributed to the same group).

Conditional residuals for cortical excitability across phases



Supplementary Tables

Supplementary Table I.1. Associations between NREM SWE (0.75-4Hz range*) and cognition.

Associations between NREM SWE (0.75-4Hz range*) and cognitive composite scores of global and domain-specific performance.

	Global performance	Memory	Attentional	Executive
	(Z-score)	(Z-score)	(Z-score)	(Z-score)
NREM SWE (0.75-4Hz)	$F_{1,55} = 0.42$ $p = 0.52$	$F_{1,55} = 1.42$ $p = 0.24$	$F_{1,55} = 0.18$ $p = 0.67$	$F_{1,55} = 1.74$ $p = 0.19$
Age	$F_{1,55} = 2.72$ $p = 0.11$	$F_{1,55} = 4.40$ $p = 0.04$ $R^2_{\beta^*} = 0.07$	$F_{1,55} = 3.34$ $p = 0.07$	$F_{1,55} = 0.19$ $p = 0.67$
Sex	$F_{1,55} = 0.02$ $p = 0.89$	$F_{1,55} = 0.29$ $p = 0.59$	$F_{1,55} = 0.13$ $p = 0.72$	$F_{1,55} = 0.04$ $p = 0.85$
Education	$F_{1,55} = 4.59$ $p = 0.04$ $R^2_{\beta^*} = 0.08$	$F_{1,55} = 0.08$ $p = 0.78$	$F_{1,55} = 2.99$ $p = 0.09$	$F_{1,55} = 4.81$ $p = 0.03$ $R^2_{\beta^*} = 0.08$

Associations between NREM SWE (0.75-4Hz range*) and cognitive composite scores of global and domain-specific performance, accounting for global and region-specific brain integrity markers.

NREM SWE (0.75-4Hz)	$F_{1,52} = 0.49$ $p = 0.49$	$F_{1,52} = 0.20$ $p = 0.66$	$F_{1,52} = 0.14$ $p = 0.71$	$F_{1,52} = 1.29$ $p = 0.26$
Age	$F_{1,52} = 3.36$ $p = 0.07$	$F_{1,52} = 5.26$ $p = 0.03$ $R^2_{\beta^*} = 0.09$	$F_{1,52} = 3.23$ $p = 0.08$	$F_{1,52} = 0.59$ $p = 0.45$
Sex	$F_{1,52} = 0.03$ $p = 0.87$	$F_{1,52} = 0.08$ $p = 0.78$	$F_{1,52} = 0.06$ $p = 0.82$	$F_{1,52} = 0.02$ $p = 0.88$
Education	$F_{1,52} = 4.72$	$F_{1,52} = 0.71$	$F_{1,52} = 2.57$	$F_{1,52} = 4.52$

	$p = 0.03$	$p = 0.40$	$p = 0.12$	$p = 0.04$
	$R^2_{\beta^*} = 0.08$			$R^2_{\beta^*} = 0.08$
Region-specific GM volume	$F_{1,52} = 0.17$	$F_{1,52} = 2.41$	$F_{1,52} = 0.03$	$F_{1,52} = 0.01$
	$p = 0.68$	$p = 0.13$	$p = 0.85$	$p = 0.92$
Region-specific A β burden	$F_{1,52} = 0.14$	$F_{1,52} = 1.66$	$F_{1,52} = 0.01$	$F_{1,52} = 0.11$
	$p = 0.71$	$p = 0.20$	$p = 0.91$	$p = 0.74$
Region-specific Tau burden	$F_{1,52} = 0.53$	$F_{1,52} = 0.79$	$F_{1,52} = 0.67$	$F_{1,52} = 1.14$
	$p = 0.47$	$p = 0.38$	$p = 0.42$	$p = 0.29$

* For simplicity, results are presented for the entire range of SWE, *i.e.* lower (0.75-1Hz) and higher (1.25-4Hz) ranges combined, but the statistical outcomes are the same when considering both ranges separately.

Supplementary Table I.2. Associations between the difference in cortical excitability values between the last and the first TMS-EEG sessions (S5–S1) and cognitive composite scores of global and domain-specific performance, adjusted for age, sex, and education. Statistical outputs of Generalized Linear Mixed Models with cognitive scores as dependent measures, accounting for their respective data distribution profiles. $R^2_{\beta^*}$ correspond to semi-partial R^2 in GLMMs.

	Global performance	Memory	Attentional	Executive
	(Z-score)	(Z-score)	(Z-score)	(Z-score)
TEP slope (S5-S1)	$F_{1,55} = 2.90$	$F_{1,55} = 0.10$	$F_{1,55} = 1.31$	$F_{1,55} = 4.75$
	$p = 0.09$	$p = 0.76$	$p = 0.26$	$p = 0.03$
				$R^2_{\beta^*} = 0.08$
Age	$F_{1,55} = 5.27$	$F_{1,55} = 2.85$	$F_{1,55} = 5.09$	$F_{1,55} = 1.83$
	$p = 0.03$	$p = 0.10$	$p = 0.03$	$p = 0.18$
	$R^2_{\beta^*} = 0.10$		$R^2_{\beta^*} = 0.08$	
Sex	$F_{1,55} = 0.04$	$F_{1,55} = 0.01$	$F_{1,55} = 0.03$	$F_{1,55} = 0.18$
	$p = 0.85$	$p = 0.92$	$p = 0.87$	$p = 0.67$
Education	$F_{1,55} = 5.16$	$F_{1,55} = 0.09$	$F_{1,55} = 3.25$	$F_{1,55} = 5.49$
	$p = 0.03$	$p = 0.77$	$p = 0.08$	$p = 0.02$
	$R^2_{\beta^*} = 0.09$			$R^2_{\beta^*} = 0.09$

Supplementary Table I.3. Associations between CEP and executive performance, after accounting for brain structural integrity markers in the left superior frontal gyrus (SFG, TMS target stimulation site). Statistical outputs of Generalized Linear Mixed Models with executive composite scores as dependent measure, accounting for its data distribution profile. $R^2_{\beta^*}$ corresponds to semi-partial R^2 in GLMMs.

	Executive (Z-score)
	$F_{1,52} = 8.99$
CEP	$p = 0.004$ $R^2_{\beta^*} = 0.15$
Age	$F_{1,52} = 3.66$ $p = 0.06$
Sex	$F_{1,52} = 0.55$ $p = 0.46$
Education	$F_{1,52} = 6.84$ $p = 0.01$ $R^2_{\beta^*} = 0.12$
Left SFG GM volume	$F_{1,52} = 0.09$ $p = 0.76$
Left SFG A β burden	$F_{1,52} = 0.34$ $p = 0.56$
Left SFG Tau burden	$F_{1,52} = 0.96$ $p = 0.33$

Supplementary Table I.4. Masks of domain-specific brain regions computed with Automated Anatomical Labeling (AAL2) atlas. The table lists the anatomical brain regions used to create the mask for the executive function (Collette & Linden, 2002; Niendam et al., 2012), memory function (Jeong, Chung, & Kim, 2015; Rugg & Vilberg, 2013), and attentional function (Corbetta & Shulman, 2002; Fan, McCandliss, Fossella, Flombaum, & Posner, 2005). Names in *italic* refer to the labels of the regions in AAL2 atlas (<http://www.gin.cnrs.fr/en/tools/aal-aal2/>).

Memory function	Attentional function	Executive function
Medial superior frontal (<i>Frontal_Sup_Medial</i>)	Superior frontal (<i>Frontal_Sup_2</i>)	Superior frontal (<i>Frontal_Sup_2</i>)
Medial orbitofrontal (<i>Frontal_Med_Orb</i>)	Middle frontal (<i>Frontal_Mid_2</i>)	Medial superior frontal (<i>Frontal_Sup_Medial</i>)
Posterior cingulate (<i>Cingulate_Post</i>)	Inferior frontal, pars triangularis (<i>Frontal_Inf_Tri</i>)	Middle frontal (<i>Frontal_Mid_2</i>)
Hippocampus (<i>Hippocampus</i>)	Superior parietal (<i>Parietal_Sup</i>)	Inferior frontal, pars opercularis (<i>Frontal_Inf_Oper</i>)
Parahippocampal (<i>ParaHippocampal</i>)	Inferior parietal (<i>Parietal_Inf</i>)	Inferior frontal, pars triangularis (<i>Frontal_Inf_Tri</i>)
Angular (<i>Angular</i>)	Anterior cingulate (<i>Cingulate_Ant</i>)	Inferior frontal, pars orbitalis (<i>Frontal_Inf_Orb_2</i>)
Precuneus (<i>Precuneus</i>)	Superior temporal (<i>Temporal_Sup</i>)	Medial orbitofrontal (<i>Frontal_Med_Orb</i>)
	Thalamus (<i>Thalamus</i>)	Anterior cingulate (<i>Cingulate_Ant</i>)
	Fusiform (<i>Fusiform</i>)	Superior parietal (<i>Parietal_Sup</i>)
	Precentral (<i>Precentral</i>)	Inferior parietal (<i>Parietal_Inf</i>)
	Postcentral (<i>Postcentral</i>)	

