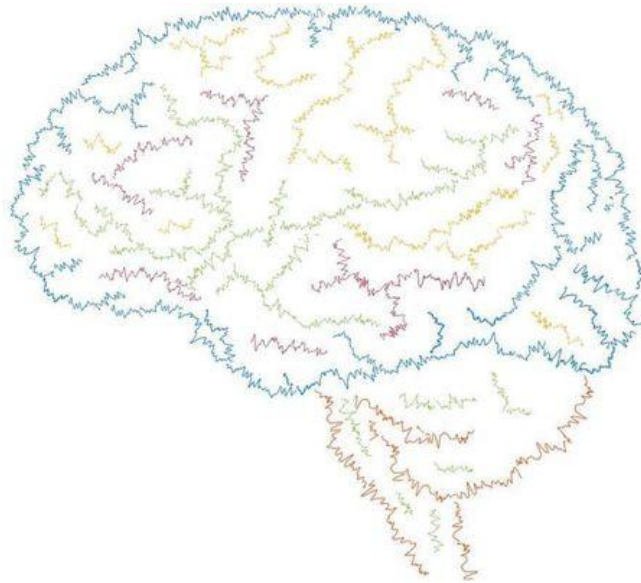


**INVESTIGATING THE LINKS BETWEEN SLEEP
MICROSTRUCTURE, COGNITION AND PET BIOMARKERS OF
ALZHEIMER'S DISEASE NEUROPATHOLOGY**



Daphne Chylinski

Thesis submitted for the academic degree of

Doctor in Psychological Sciences

2022

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NEUROPATHOLOGY**

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Master of Sciences, Université Libre de Bruxelles, 2015

A Dissertation Submitted in Partial Fulfilment of the Requirements for the
Academic Degree of

Doctor in Psychological Sciences

In the Faculty of Psychology, Speech Therapy and Educational Sciences

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UNIVERSITY OF LIÈGE

March 2022

“If the brain were so simple that we could understand it,
we would be so simple that we couldn’t.”

E. Pugh

Acknowledgements

Firstly, I would like to express my gratitude to my supervisor, Gilles Vandewalle, for giving me the opportunity to do a PhD at the Cyclotron Research Centre. Thank you for believing in me, for your availability, and your guidance throughout this journey, for pushing me out of my comfort zone. I'm particularly grateful to you for your support and help during the writing process. My thanks also go to my co-supervisor, Fabienne Collette, for the interesting discussions about cognition. I also thank the both of you for giving me the comfort of calmly finishing my dissertation.

I would also like to acknowledge the members of the jury, Jean-Marc Lina, Christian Cajochen, Steve Majerus, Pierre Maquet, and Christophe Phillips, for accepting to review my work and debate it during the defence. Thank you for your time and efforts in evaluating this dissertation, and for the opportunity to discuss it together.

My particular thanks go to Jean-Marc Lina and Julie Carrier, for the chance to work together on such an interesting topic. I'm deeply grateful to Jean-Marc, for his availability and never failing enthusiasm, and his questions that were always on point. It was a real pleasure to collaborate. I would also like to thank Pierre Maquet, for his relevant comments, his insights, and the way he makes people feel in the radiating halo of his knowledge (though the mind's night is only darker when he leaves).

I want to give a particular thank you to my former colleagues on the COFITAGE study, Maxime and Justinas. The shared time spent in acquisition is a moment I go back to with nostalgia. Thank you Justinas for being so welcoming and supporting. Maxime, thank you for everything we shared, for all that you taught me, and for all your support.

I also thank my partners in crime, Catherine and Nora, for introducing to south-belgian living the capital girl that I was. Thank you for everything, the breaths of (not so) fresh air, all the singing and visits in our beautiful Ardennes. It is a love based on giving and receiving, as well as having and sharing.

I am also deeply grateful to Vincenzo, for taking me under his wings, and to him and Christina, for their friendship and support. A big thank you to all my colleagues and friends from the CRC, Adrien, Jessica, Camille, Mathilde, Michele, Martin, Zoltan, Florence, Maëlle, Marine, by order of appearance, for the amazing time we spent together, the ups and downs,

the shared victories and defeats. I truly felt like being part of a big family, and I sincerely regret not being able to spend more time with you in the second half of my PhD. I'm particularly grateful to Martin, for all his help with coding, you saved me from the many concussions I would have had from banging my head on my screen if it wasn't for you.

I also want to thank my former master thesis co-supervisor and friend, Hichem, who contributed greatly to my interest in the brain. Thank you for everything you taught me, in research as well as in the clinic, and for all the oenology lessons.

My gratitude also goes towards my family, particularly my sister, who did an excellent job at being my gaoler during the final part of my writing, when I was well embarked on the end-of-thesis emotional roller-coaster. My thanks also go to my mum, for always caring for me and believing in me. My biggest thanks to Przemek, the companion of my oh-so-many adventures, for moving yet another mountain with me. Your strength and will inspire me every day.

As someone very wise once wrote: "It's a dangerous business, going out your door. You step onto the road, and if you don't keep your feet, there's no knowing where you might be swept off to." I'm very grateful to all of you who were a part of the last four years of my life, you greatly contributed to the exciting adventure that this PhD was.

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List of abbreviations

A β	amyloid-beta
AD	Alzheimer's disease
AHI	apnoea/hypopnoea index
ApoE	apolipoprotein E
APP	amyloid precursor protein
CSF	cerebrospinal fluid
DWI	diffusion-weighted imaging
EEG	electroencephalogram
EMG	electromyogram
EOG	electrooculogram
GABA	γ -aminobutyric acid
GLMM	generalised linear mixed model
ISF	interstitial fluid
LC	locus coeruleus
MCI	mild cognitive impairment
NFT	neurofibrillary tangle
NREM	non-rapid eye movement (sleep)
OSA	obstructive sleep apnoea
REM	rapid eye movement (sleep)
SDB	sleep disordered breathing
SCN	suprachiasmatic nucleus
SHY	synaptic homeostasis hypothesis
SUV _R	standardised uptake value ratio
SW	slow waves
SWA	slow wave activity
SWE	slow wave energy
SWS	slow wave sleep

SUMMARY

Sleep is an essential physiological state, present throughout the animal kingdom, and known to play a key role for both health and cognition in humans. Importantly, sleep is known to undergo a series of changes across the human lifespan, while an increasing amount of evidence is showing these age-related alterations in sleep are linked to cognitive and cerebral pathophysiological changes.

In the present dissertation, we will first introduce the organisation of sleep and describe models contributing to the regulation of the sleep-wake cycle, as well as the reported functions of sleep. We will then present some main aspects of sleep microstructure at the level of the electroencephalogram (EEG), and the relationship between sleep and the changes at the cognitive and cerebral level during the ageing process, including pathological aging and more particularly Alzheimer's disease (AD).

Crucially, recent literature is pointing towards a bidirectional relationship between sleep and the hallmarks of AD pathophysiology, i.e. the accumulation of hyperphosphorylated tau and amyloid- β , and neurodegeneration. Poorer sleep quality, daytime sleepiness and lower slow wave generation have all been linked to higher deposition of those proteins, as well as to poorer cognitive performance. Conversely, sleep has been suggested to play a key role in the cleaning of brain metabolite waste. The general aim of the present dissertation was to investigate, in a healthy population, the microstructural characteristics of sleep which are associated to increased risk factors for the future development of AD, in the form of its increased biomarkers.

In the methods section, we first we assessed the sleep stage scoring inter-scorer variability in data collected in a sample of healthy older individuals, by comparing two visual and one automated sleep scoring. We report higher inter-expert variability than what is usually presented in the literature, particularly for the distinction between non-rapid eye movement (NREM) sleep stage 2 and NREM3. In the second methodological paper, we address the validity of an automatic arousal detection algorithm developed in the lab. Despite the higher arousal count delivered by the automatic detector, we find good agreement with visual scoring of arousal events, reflected by high Cohen's kappa values. Together, these results indicate that, even though visual scoring remains the gold standard for sleep scoring, whether for sleep stage or for the detection of events such as arousals, automatic algorithms shows similar agreement

with human scoring than visual scorers among themselves, while they offer a fast and reliable way of scoring which remains constant across recordings and thus favours time-efficient standardisation.

In the first results chapter, we examine the potential heterogeneity in spontaneous sleep arousals and their association with amyloid- β , and cognition. We find that, contrary to what may be most commonly accepted, arousals are diverse sleep phenomena, characterised by different oscillatory compositions, and are differently associated with early cortical amyloid- β and cognition, particularly attentional function. These results suggest that spontaneous arousals may actively contribute to the beneficial functions of sleep.

In the second results section, we investigate how brain tissue microstructure as assessed through MRI measures of neurite orientation dispersion and density over a set of frontal grey matter regions is related to the generation of sleep slow waves. We find that both neurite density and orientation are associated with slow wave characteristics. Our results imply that frontal grey matter density of neurite plays a crucial role for neuronal synchronisation, while the branching complexity of the dendritic arborisation contributes to the rate of slow wave occurrence.

In the third results chapter, we assessed the precise co-occurrence between slow waves and spindles, two key waveforms of NREM sleep, and show that their relative uncoupling is associated to a higher amyloid- β burden over the medial prefrontal cortex, as well as with a memory worsening over a 2 years longitudinal follow-up, therefore indicating that an altered coupling of sleep microstructure elements key to its memory function is linked to poorer brain integrity and predictive of a memory decline.

The last section of this work is devoted to the general discussion of the results presented in each respective paper, integrating the insight gained from each section. We then finish by putting our results in perspective for future research.

We hope that this work will be of interest to the broad community of sleep research, and that it will be part of an increasing research on the dynamic aspects of sleep microstructure. We wish the reader a pleasant read.

RÉSUMÉ

Le sommeil est un état physiologique essentiel, présent dans tout le règne animal, et connu pour jouer un rôle clé à la fois pour la santé et la cognition chez l'homme. Il est important de noter que le sommeil subit une série de changements tout au long de la vie humaine, tandis qu'un nombre croissant de preuves montre que ces altérations du sommeil liées à l'âge sont associées à des changements physiopathologiques cognitifs et cérébraux.

Dans la présente thèse, nous introduirons d'abord l'organisation du sommeil et décrirons les modèles contribuant à la régulation du cycle veille-sommeil, ainsi que les fonctions rapportées du sommeil. Nous présenterons ensuite quelques aspects principaux de la microstructure du sommeil au niveau de l'électroencéphalogramme (EEG), et la relation entre le sommeil et les changements au niveau cognitif et cérébral au cours du processus de vieillissement, y compris le vieillissement pathologique et plus particulièrement la maladie d'Alzheimer (MA).

De manière cruciale, la littérature récente indique une relation bidirectionnelle entre le sommeil et les caractéristiques de la physiopathologie de la MA, c'est-à-dire l'accumulation d'amyloïde- β et de tau hyperphosphorylé, et la neurodégénérescence. Une mauvaise qualité de sommeil, une somnolence diurne et une diminution de la production d'ondes lentes ont toutes été liées à un dépôt plus important de ces protéines, ainsi qu'à de moins bonnes performances cognitives. Inversement, il a été suggéré que le sommeil joue un rôle clé dans le nettoyage des déchets métaboliques du cerveau. L'objectif général de la présente thèse était d'étudier, dans une population saine, les caractéristiques microstructurelles du sommeil qui sont associées à des facteurs de risque accrus pour le développement futur de la MA, sous la forme d'une augmentation de ses biomarqueurs.

Dans la section sur les méthodes, nous avons évalué la variabilité inter-experts du scorage des stades du sommeil dans des données recueillies auprès d'un échantillon de personnes âgées en bonne santé, en comparant deux scorages visuelles et un scorage automatique du sommeil. Nous rapportons une variabilité inter-expert plus élevée que ce qui est habituellement rapporté dans la littérature, en particulier pour la distinction entre le stade 2 du sommeil sans mouvements oculaires non rapides (NREM) et le NREM3. Dans le second article méthodologique, nous nous penchons d'abord sur la validité d'un algorithme de détection automatique de micro-réveils développé dans le laboratoire. Malgré le nombre de

micro-réveils plus élevé fourni par le détecteur automatique, nous constatons une bonne concordance avec le scorage visuel des micro-réveils, reflétée par des valeurs élevées du kappa de Cohen. L'ensemble de ces résultats indique que, même si le scorage visuel reste l'étalon-or pour le scorage du sommeil, que ce soit pour le scorage des stades de sommeil ou pour la détection d'événements tels que les micro-réveils, les algorithmes automatiques présentent une concordance similaire avec la notation humaine que les évaluateurs visuels entre eux, tout en offrant une méthode de scorage rapide et fiable qui reste constante d'un enregistrement à l'autre et favorise donc une standardisation efficace en termes de temps.

Dans le premier chapitre des résultats, nous examinons l'hétérogénéité potentielle des micro-réveils spontanés durant le sommeil et leur association avec l'amyloïde- β et la cognition. Nous constatons que, contrairement à ce qui est communément admis, les micro-réveils constituent une catégorie hétérogène de phénomènes durant le sommeil, caractérisés par différentes compositions oscillatoires, et sont différemment associés à la charge corticale en amyloïde- β précoce et à la cognition, en particulier à la fonction attentionnelle. Ces résultats suggèrent que les micro-réveils spontanés peuvent contribuer activement aux fonctions bénéfiques du sommeil.

Dans la deuxième section des résultats, nous étudions comment la microstructure du tissu cérébral, évaluée par des mesures IRM de la dispersion de l'orientation ainsi que de la densité des neurites sur un ensemble de régions de la matière grise frontale, est liée à la génération des ondes lentes de sommeil. Nous constatons que la densité et l'orientation des neurites sont associées aux caractéristiques des ondes lentes. Nos résultats impliquent que la densité des neurites dans la matière grise frontale joue un rôle crucial dans la synchronisation neuronale, tandis que la complexité de la ramification de l'arborisation dendritique contribue à la fréquence d'apparition des ondes lentes.

Dans le troisième chapitre de résultats, nous avons évalué la cooccurrence précise entre les ondes lentes et les fuseaux, deux formes d'ondes clés du sommeil NREM, et nous montrons que leur désynchronisation relative est associée à une charge amyloïde- β plus élevée dans le cortex préfrontal médian, ainsi qu'à une détérioration de la mémoire sur un suivi longitudinal de 2 ans, indiquant ainsi qu'un couplage altéré des éléments de la microstructure du sommeil essentiels à sa fonction de mémoire est lié à une moins bonne intégrité cérébrale et prédit un déclin de la mémoire.

La dernière section de ce travail est consacrée à la discussion générale des résultats présentés dans chaque article respectif, en intégrant les connaissances acquises dans chaque section. Nous terminons ensuite en mettant nos résultats en perspective pour les recherches futures.

Nous espérons que ce travail présentera un intérêt pour la vaste communauté de la recherche sur le sommeil, et qu'il s'inscrira dans le cadre d'une recherche croissante sur les aspects dynamiques de la microstructure du sommeil. Nous souhaitons au lecteur une agréable lecture.

Theoretical framework

Chapter I: Sleep – General introduction

Sleep is a fundamental physiological state, ubiquitous throughout the animal kingdom. At the behavioural level, sleep can be defined as a reversible state of reduced vigilance and response to the environment, characterised by physical quiescence. It has to be pointed out, though, that sleep is not a uniform phenomenon, and that its different substates are highly variable in terms of those defining features (Peigneux, Urbain, & Schmitz, 2011). In the present chapter, we will start by reviewing the main architecture of sleep (i.e. its macrostructure) and how different states alternate across the night, with the main electroencephalogram (EEG) characteristics. We will then elaborate on the theory of how sleep and wakefulness are regulated, so that they give rise, in human adults, to consolidated periods of ~16h of wakefulness, followed by ~8h of sleep. Then, we will describe the current knowledge about the neural circuitry underlying these different states. We will also challenge the once dominant view that sleep constitutes a global, whole-brain phenomenon by reviewing its local aspects. Further, we will briefly overview the genetic regulation of sleep. In the following section, we will review the main functions that have been put forward regarding the reason as to why we sleep. Finally, we will review the main changes that sleep undergoes across the lifespan, focussing on the ageing process.

Sleep macrostructure

The discovery of electroencephalography (EEG) by Hans Berger in 1929 (Karbowski, 1990) has opened a new horizon in the characterisation and understanding of sleep, by exposing the heterogeneity of the brain's electrical activity during sleep. Indeed, several physiological parameters allowed to distinguish between two states of sleep: paradoxical sleep or Rapid Eye Movements (REM) sleep, and Non-REM (NREM) sleep, which can be found in most mammals and in birds, while recent reports imply even reptiles, fish and invertebrates exhibit two-stage sleep (Siegel, 2005, 2008; Yamazaki et al., 2020).

Though it is sometimes possible to define sleep stages based on behavioural criteria, like jerky eye movements in REM sleep, sleep research heavily relies on EEG recordings, a powerful non-invasive techniques allowing to study electrophysiological dynamics of the brain (Cohen, 2017). The EEG allows us, through electrodes placed on the scalp, to record brain electrical fields, which are the resultant of electrochemical signalling between neurons, mostly membrane hyper- or depolarisation of cortical pyramidal neurons from deep cortical layers (Cohen, 2017; Kirschstein & Köhling, 2009).

A typical EEG setup consists of electrodes placed on the scalp according to the international 10-20 system (Carden, 2009), which is derived from four landmark points (the nasion, or intersection between the frontal and nasal bones; the inion, or prominent part of the occipital bone; and the left and right mastoids). The electrodes are then placed at either 10 or 20% of the total front-to-back and left-to-right (from left to right mastoid) distance, the intersecting point between those two lines corresponding to the central electrode (see **figure 1**). Conventionally, electrodes placed on the left side of the scalp are referred to with odd numbers, while electrodes on the right are attributed even numbers.

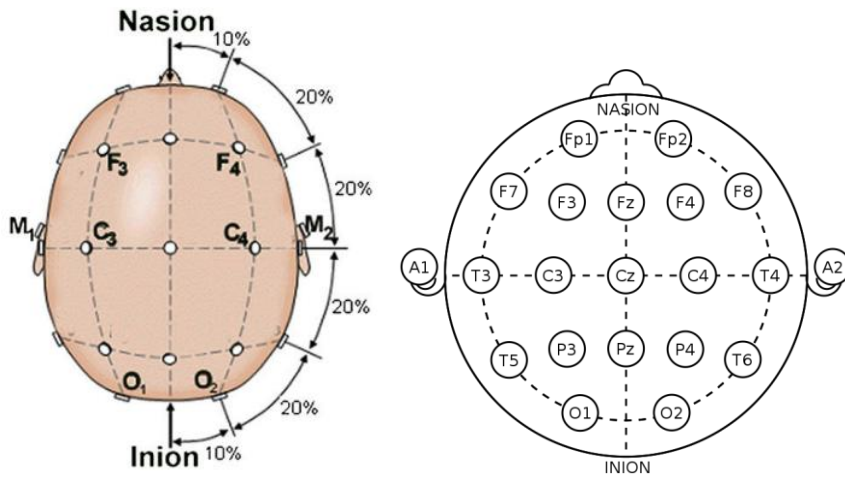


Figure 1. The 10-20 electrode placement system – electrodes are placed at 10 or 20% of the front-to-back and left-to-right distance. Reproduced with permission from (Iber, Ancoli-Israel, Chesson, & Quan, 2007)

Relatively early on in the emergence of sleep medicine and research, the need for a standardised way of scoring sleep (i.e. attributing a sleep stage to an EEG segment), arose. Several attempts to propose guidelines to score sleep, first in 20s windows, then in 30s windows (called scoring windows or epochs) were done over the years, first by Rechtschaffen & Kales (Rechtschaffen & Kales, 1968), then by the American Academy for Sleep Medicine (AASM) (Berry et al., 2017; Berry et al., 2015; Iber et al., 2007). These guidelines, though they do not abolish inter- and intra-scorer variability, constitute a way of standardising sleep scoring and thus increase comparability between studies and centres (see experimental protocol section for more discussion on the subject).

In this section, we will describe the different stages across which one goes over the sleep-wake cycle, with their main EEG and physiological characteristics, as well as their distribution across the night.

Wakefulness

The EEG patterns seen during wakefulness include a diversity of brainwaves which are dependent upon several factors, such as the time elapsed since the last sleep episode, or the content and behavioural characteristics of the waking period, but this is outside the scope of the present thesis. In general, the wakefulness EEG is characterised by low amplitude, high

voltage waves (see **figure 2**). In sleep research and medicine, stage wakefulness (W) ranges from complete alertness to the early stages of drowsiness. With open eyes, the EEG is characterised mainly by so-called beta (14-30Hz) and alpha (8-12Hz) frequencies. With closed eyes, this translates to predominant alpha rhythm in most individuals, particularly over the occipital cortex (about 10% of people do not produce it, and their occipital wake EEG is similar with open and closed eyes (Iber et al., 2007)). The electrooculogram (EOG) can present rapid non-stereotyped eye movements (0.5-2Hz), blinks or reading movements. Progressively, with increasing drowsiness, blinks are gradually replaced by slow eye movements. The electromyogram (EMG) is of variable amplitude, but usually displays relatively high chin muscular tone (the chin being the most typical location where EMG electrodes are placed).

NREM

NREM is an inhomogeneous sleep state, and can be further divided into sub-stages depending on the preponderance of slow waves on the EEG, which roughly reflects the depth of sleep. NREM is accompanied by a decrease in body temperature, a slowing of breathing and heart rate and a reduced response to external stimuli. Originally, Rechtschaffen and Kales distinguished four stages of NREM (S1-S4), but in 2007 the American Academy of Sleep Medicine simplified the system and proposed a scoring system distinguishing only three NREM stages (N1, N2, N3, where N3 is the combination of stage S3 and S4).

Stage N1 is characterised by low amplitude, mixed frequencies, a reduction of alpha rhythms (in people who generate it), which is replaced by low amplitude, mixed frequencies (predominantly theta 4-7Hz frequencies) over more than 50% of the scoring epoch. Slow eye movements characterise the EOG. Occasional vertex sharp waves - well delineated “pointy” waves of less than 500ms, largest over central regions - can be seen. Stage N2 features two characteristic EEG signatures, that is spindles – waxing and waning waveforms of 11-16Hz - and K complexes – well-delineated larger waves composed first of a sharp negative peak followed by a positive one, for which the total duration is equal or higher than 500ms. Stage N3 is richer in slow oscillations, and is composed of >20% of slow waves. The originally differentiated fourth stage was characterised by slow oscillations occupying more than 50% of the scoring epoch. Both slow waves and spindles will be cover in more details in chapter 2.

Interestingly, arousability is related to the depth of the sleep stage: it is much easier to arouse an individual in N1, and requires much stronger stimulation to arouse them from N3 when the EEG shows predominance of slow waves.

REM

REM sleep is characterised by rapid, desynchronised, lower amplitude EEG activity, reminiscent of the activity that can be recorded during wakefulness, with typically theta and alpha activity dominating the EEG background, and the occasional appearance of “sawtooth waves” – sharply delineated waves of 2-6Hz. Muscle atonia and bursts of stereotyped rapid eye movements are also characteristic of this sleep stage. REM sleep is composed of two relatively distinct neural substates, that is phasic and tonic REM periods. Phasic REM can be seen as a more active period, and is characterised by bursts of rapid eye movements, as well as sawtooth waves and irregular respiratory and cardiac activity. Tonic REM periods, on the other hand, are more quiescent phases of activity.

	EEG	EOG	EMG
WAKE			
NREM1			
NREM2			
NREM3			
Phasic REM			
Tonic REM			

Figure 2. Summary of the main electroencephalogram (EEG) patterns observed in each sleep stage, as well as typical electrooculogram (EOG) and electromyogram (EMG) activity for each stage.

Sleep cycles

Over the course of a night of sleep, those different sleep stages (N1-N2-N3-REM) will succeed to form a sleep cycle, which will be repeated several times during the sleeping

period. Typically, one cycle lasts about 90 minutes, and a regular period of night sleep will be composed of around 5 sleep cycles. The classical alternating sequence of the above-mentioned sleep stages can be represented by a sleep hypnogram (**figure 3**).

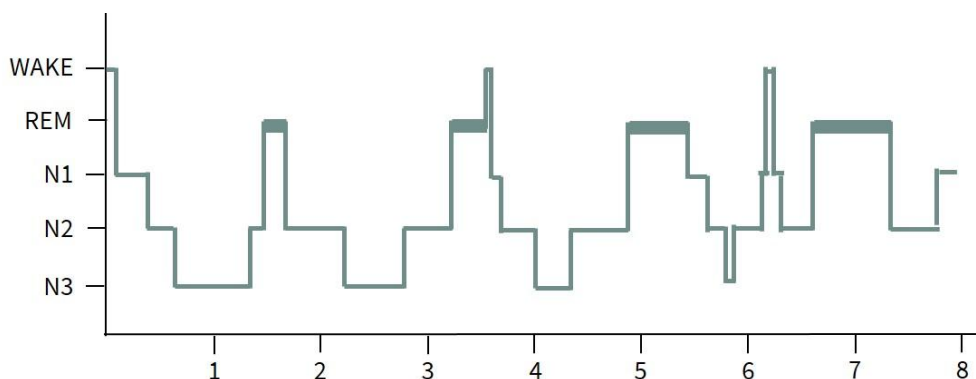


Figure 3. A sleep hypnogram, representing the temporal succession of sleep stages across the night. A sleep cycle is composed of the different NREM stages followed by a REM period.

Classically used sleep parameters in sleep research and sleep medicine derived from EEG recordings include sleep onset latency, defined as the time between lights off and the first epoch scored as N2 (sometimes N1); sleep efficiency, or the ratio between the time spent asleep and the time spent in bed (between lights off and lights on); wake after sleep onset (WASO) or the time spent awake after the first sleep onset; number of awakenings; duration or percentage of sleep time spent in each sleep stage; and latency to N3 and REM, defined as the time difference between sleep onset and the first epoch spent in N3/REM sleep stage.

Importantly, the cyclic alternation between sleep stages throughout the night does not merely constitute a repetition of identical cycles, but rather is impacted by processes from the beginning to the end of the sleeping period (Halasz & Bodizs, 2012). Indeed, it was shown that across the night, depth of sleep is reduced, which is reflected in less and less time spent in N3 with less and less slow waves, manifesting the dissipation of the process S, along with an increase in the length and density of REM periods and N2 bouts from evening to morning, which are controlled by the circadian system (Czeisler, Zimmerman, Ronda, Moore-Ede, & Weitzman, 1980; Kales, Jacobson, Kales, Kun, & Weissbuch, 1967).

Interestingly, preserved architecture of sleep cycles but also of their different dynamics across the night can be a marker of pathology. While a shorter REM latency can be normal when initiating sleep in late morning hours, it does not happen at the beginning of the

night in healthy subjects (Bes, Jobert, Müller, & Schulz, 1996). Not only do sleep cycles change across the night, but also sleep stages, depending on their position within a sleep cycle, can exhibit variation. In fact, it was shown that the descending slope of a sleep cycle (from its beginning until its first deepest phase) is significantly longer than the ascending one (from its last deepest phase until its next highest stage), and also contains more stage transitions (see **figure 4**), and less stage ‘jumps’, where transitions “skip” a stage. It is thus assumed that descending and ascending slopes of sleep cycles carry different dynamic function (sleep promotion vs wake/REM promotion), which presumes functional differences between the same sleep stage on different parts of the slope, beyond their common EEG characteristics (Halasz & Bodizs, 2012).

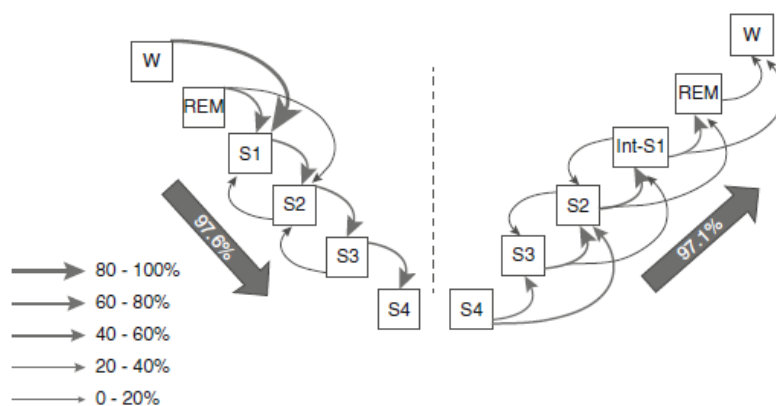


Figure 4. Representation of the distribution of stage transitions observed on the ascending and descending slopes of sleep cycles in healthy young individuals. Reproduced with permission from (Halasz & Bodizs, 2012)

Conclusion

Based on the study of EEG, a pattern of repeated alternation between different sleep states, forming a sleep cycle, has been recognised as a crucial feature of human sleep. NREM and REM sleep are fundamental units composing a sleep episode, which are defined by distinct EEG characteristics. It is important to note, however, that the EEG is merely a biomarker of an underlying state, and that it does not always reflect changes in said state. For instance, activity in the delta frequency (0.5-4Hz) band can remain low during sleep induced via benzodiazepine, while it can be high during wakefulness in post-ictal state (Scammell, Arrigoni, & Lipton, 2017). Also, it is central to understand that sleep stages represent an arbitrary reduction in sleep dynamics and in the diversity of their underlying

neurophysiological states. The scoring of sleep in fixed, 30s windows, though serving practical and time-saving purposes in sleep medicine and research, is nonetheless reductive. In the chapter about sleep microstructure, we will review in more details the graphoelements interwoven in the sleep EEG which reflect, in a more precise and reduced timeframe, the dynamics aspects of sleep.

Regulation of sleep and wakefulness

It has been proposed that the successive alternation between sleep and wakefulness is orchestrated by two independent biological mechanisms that interact and counteract each other, in order to create consolidated periods of wakefulness of ~16h followed by ~8h of sleep. Alexander Borbély first described in 1982 the two process model of sleep-wake regulation, which, though updated since with current knowledge (Borbély, Daan, Wirz-Justice, & Deboer, 2016), remains largely valid today (Borbély, 1982). In this model, he depicted the sleep-wake homeostasis, or Process S, and the circadian rhythms, known as Process C. Sleep-wake homeostasis refers to the increase in sleep pressure accumulated over the course of a period of wakefulness and its subsequent decline over the following period of sleep, while circadian rhythms term the nearly 24h mechanism that regulates our body's internal processes, amongst which hormone secretion, body temperature, immune response, and the timing and propensity of sleep (Edery, 2000; Vitaterna, Takahashi, & Turek, 2001).

Sleep-wake homeostasis can be viewed as an hourglass mechanism, which keeps track of previous sleep and wakefulness history, and builds or dissipates sleep need accordingly. It is modelled by two exponential functions: a saturating exponential function reflecting the rise of sleep pressure during wakefulness, and a declining exponential function reflecting its dissipation (see **figure 5**) (Achermann & Borbely, 2017).

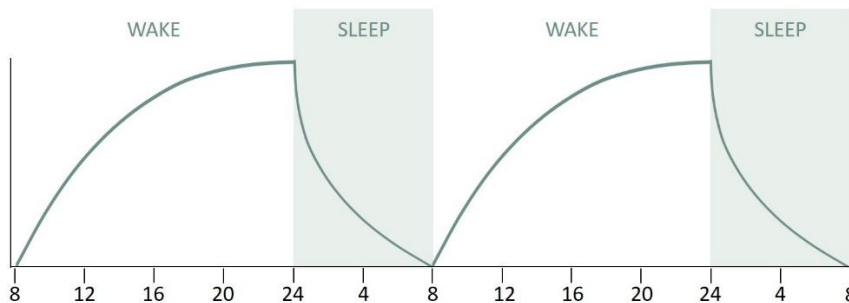


Figure 5. *Illustration of the sleep homeostasis process, one of the two sleep-wake regulatory mechanisms depicted in the theoretical model of sleep and wakefulness regulation, adapted from (Borbély, 1982). The ascending curve is a saturating exponential function and reflects the rise of sleep pressure during wakefulness, while the declining exponential function reflects its dissipation.*

While we are all aware of the detrimental consequences that a lack of sleep has at the behavioural level (decrement in cognitive performances, increased somnolence and irritability, to name just a few (Alhola & Polo-Kantola, 2007; M. Bonnet, 1985; Dijk, Duffy, & Czeisler,

1992; Killgore, 2010; Pilcher & Huffcutt, 1996), the effects of sleep deprivation can also be measured on the electroencephalogram (EEG). Generally speaking, prolonging the period of wakefulness results in a shift towards the slower frequency brain activity, mostly over the so-called theta frequency band of the EEG (4-8Hz), while during sleep, sleep need is reflected in the power in the so-called delta band of the EEG (0.5-4Hz). Slow wave sleep (SWS), highly composed of slow waves with frequency < 4 Hz, has been shown to increase as a function of previous wake duration (Webb & Agnew, 1971). Slow-wave activity (SWA), the power delivered in the delta range (0.5-4 Hz), has been suggested as a marker of process S. Interestingly, absolute SWA values show high inter-individual variability, related to conductivity of the involved tissues (scalp, skull, cerebrospinal fluid (CSF), grey and white matter (Laarne, Kauppinen, Hyttinen, Malmivuo, & Eskola, 1999)), and are largely dependent on age, and thus absolute SWA levels are not a straightforward measure of sleep homeostasis (Achermann & Borbely, 2017). Rather, it is the relative power of SWA or the relative change in SWA that is revealing, namely the difference between SWA and the rest of the frequency of the EEG or between SWA of a regular night and that observed after a challenge (sleep deprivation or restriction, nap, etc). On those grounds, wake extension and sleep deprivation protocols have shown increased SWA during the recovery night as compared to baseline (Dijk, Hayes, & Czeisler, 1993; Skorucak, Arbon, Dijk, & Achermann, 2018). Conversely, taking a nap during the day will further lower homeostatic sleep pressure and decrease the density/amplitude of slow waves produced the following night (Dijk, Beersma, & Daan, 1987). Naps taken earlier during the day will contain less SWA than naps taken later in the day (ibidem). At the molecular level, another marker of sleep homeostasis is reflected by the accumulation of hypnogenic substances, such as adenosine, in different areas of the brain (Reichert, Maire, Schmidt, & Cajochen, 2016). Adenosine extracellular concentration increases during sleep deprivation and decreases during recovery sleep (Porkka-Heiskanen et al., 1997), and caffeine has been shown to block adenosine receptors (Ribeiro & Sebastiao, 2010). In rats, extrasynaptic glutamate has been shown to gradually increase during the active state, while it decreased during Non-Rapid Eye Movement (NREM) sleep (Dash, Douglas, Vyazovskiy, Cirelli, & Tononi, 2009). In humans, one night of sleep deprivation is associated with an increase in glutamate receptor subtype 5 availability, which correlates with the increase in subjective sleepiness (Hefti et al., 2013).

Tononi and Cirelli (Tononi & Cirelli, 2003, 2006, 2014) also put forward the synaptic homeostasis hypothesis (SHY), according to which sleep is viewed as “the price to pay for

plasticity” (Tononi and Cirelli, 2014, p.12). At the cellular level, wakefulness and its inherent learning challenges result in synaptic plasticity changes and potentiation, leading to an increase in synaptic weight and an increment in energy consumption, contributing to sleep pressure. Sleep is seen as constituting a regulatory mechanism which helps restore synapses to their baseline level (see further section on the functions of sleep).

The circadian process is the internal oscillator that regulates behavioural and physiological signals with a period of about 24h and is, per contra, a sleep-independent mechanism (see **figure 6**). Three main characteristics define circadian oscillators: i) an oscillatory period approximating 24 hours (*circa- dies*), ii) their endogenous character, meaning their rhythmicity is preserved even in the absence of or in the case of changes in the environmental time cues, but is subject to iii) the influence that environmental time cues (or *zeitgebers*), modifying or resetting the rhythm’s timing/phase (Czeisler & Buxton, 2017; Gaggioni, Maquet, Schmidt, Dijk, & Vandewalle, 2014).

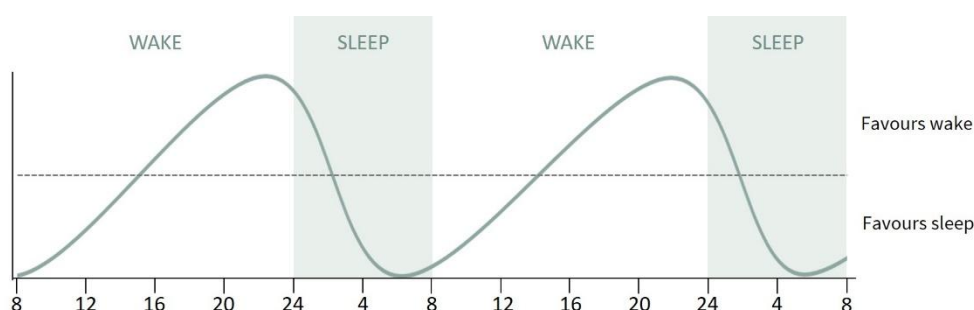


Figure 6. Illustration of the circadian process, the sleep-wake regulatory mechanisms depicted in the theoretical model of sleep and wakefulness regulation, adapted from (Borbély, 1982). The dashed line indicates the symbolic transition of the circadian signalling from favouring sleep to favouring wakefulness.

Circadian rhythmicity influencing human sleep-wake patterns has long been a subject of investigation. Seventy-two hours sleep deprivation protocols have shown that sleepiness ratings exhibit a marked circadian rhythmicity, with alternations between higher/lower subjective fatigue based on the time of day, while sleep pressure is continuously increasing (Akerstedt and Froberg, 1977, from Borbély, 1982). The conductor of our endogenous circadian rhythms, i.e. the circadian master clock, is located in the suprachiasmatic nuclei

(SCN) of the hypothalamus. Damaging the SCN leads to the suppression of a variety of circadian rhythms, such as in drinking and locomotor activity, or neuroendocrine regulation (Moore and Eichler, 1972; Rusak and Zucker, 1979; Stephan and Zucker, 1972; from Eastman et al., 1984). Circadian rhythms oscillate with a period of about 24h that is actually on average slightly longer in most people (Czeisler et al., 1999). This means that were we not resynchronising each and every day our internal clock with the outside world, for it to fit with the day/night cycle of the earth's rotation, we would continuously delay our bed and wake times and would end up going completely around the clock. Such external *zeitgebers* (time giver in German), include our social and physical activity, our meals, though the most important of all is light (Duffy, Kronauer, & Czeisler, 1996; Roenneberg & Mellow, 2007). Disorders of the sleep-wake cycle in which patients are unable to receive or respond sufficiently to timekeeping signals, result usually in a daily rhythm slightly longer than 24h, where everyday activities such as eating, sleeping and waking is each day somewhat delayed. Non-24-hour sleep-wake rhythm disorder (N24SWD), a disorder previously referred to as free-running disorder, is a type of pathology commonly seen for instance in most blind individuals lacking light perception, but also observed though more rarely, in sighted individuals (Abbott, 2019).

Several markers of the circadian process have been identified. Among them, melatonin, a hormone synthesised from serotonin and produced in the pineal gland, usually starts being secreted a few hours before habitual bedtime, peaks around the middle of the night and then decreases before habitual wake time (Aulinas, 2019). Interestingly, one main function of melatonin is to regulate the phase of circadian rhythms through direct connections to the SCN (Cajochen, Kräuchi, & Wirz-Justice, 2003; Gillette & Tischkau, 1999). As a hormone secreted during the dark phase, melatonin is also critical for the regulation of physiological changes related to seasons, e.g. the patterns showed an increase duration (see **figure 7**) (Cardinali & Pevet, 1998) and higher levels (Morera & Abreu, 2006) of melatonin secretion during winter nights, though other studies failed to replicate this, only finding a seasonal phase delay in melatonin secretion (Illnerová, Zvolsky, & Vaněček, 1985). Evening usage of electronic device emitting blue-enriched light has been shown to increase alertness, delay the phase of melatonin secretion, and thus increase sleep latency (Cajochen, 2007; Cajochen et al., 2011; Chang, Aeschbach, Duffy, & Czeisler, 2015). Cortisol, a stress hormone produced in the adrenal cortex has its secretion peak before wake time, presumptively in order to prepare a state of readiness of the system to face up to the multiple challenges and demands of the

waking period, while cortisol levels are lowest around sleep onset (Czeisler & Buxton, 2017). Also, core body temperature follows a well-studied circadian oscillation, which is almost the mirror reflection of that of melatonin secretion (Czeisler & Buxton, 2017; Wyatt, Ritz-De Cecco, Czeisler, & Dijk, 1999).

Critically, in mammals, the circadian regulation consists of a network of interconnected transcriptional-translational feedback loops, involving complex genetic and molecular mechanisms present in most, if not all, cells of the body (Blum, Bell, & Wu, 2018) (see chapter on genetic regulation of sleep). It has to be noted though, that while circadian markers and the origin of the circadian signalling have been relatively well studied, our understanding of its nature or the way by which the SCN interacts with the rest of the body remains surprisingly poor (Turek, 1994).

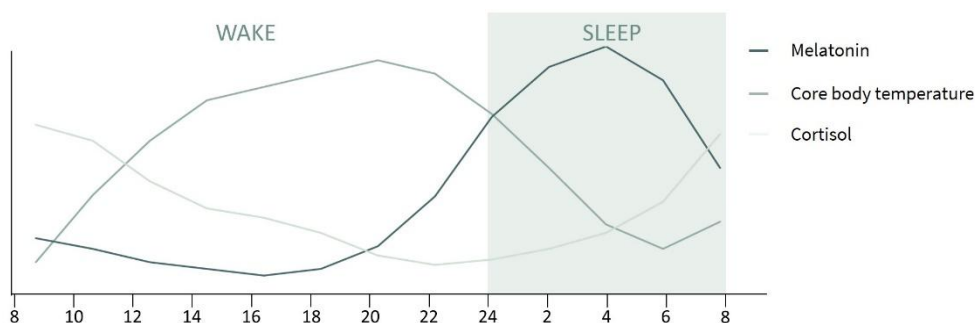


Figure 7. Illustration of the time course of melatonin and cortisol concentration and core body temperature variations across the sleep-wake cycle for a person usually sleeping from ~11PM to ~7AM

Thus, circadian and homeostatic processes both independently influence but also interact with each other in order to regulate not only time and propensity to sleep, but also waking behaviour. During a regular day, the increase of homeostatic sleep pressure accumulated since awakening is compensated by an increasing wake promoting signal from the circadian system, which peaks about 2 hours before habitual bedtime, a moment which has been called the circadian wake-maintenance zone. Conversely, a few hours before usual wake time, when the homeostatic sleep pressure has been almost entirely dissipated, the circadian signalling putatively reaches its lowest level, which constitutes a sleep promoting signal, thus ensuring the maintenance of sleep, a moment which is referred to as the sleep-promoting zone. A reflection of this sleep promoting signal can be sensed in the circadian variation in REM sleep amount with more REM sleep towards the end of the night. A striking example of the interaction between sleep homeostasis and circadian rhythmicity can be found in sleep

deprivation protocols, where better performances are observed during the wake maintenance zone than earlier during the day, in spite of the longer time spent awake (Shekleton et al., 2013), while performance undergoes a sharp decrement if wakefulness is prolonged beyond habitual sleep time because of the combined influence of high sleep need and the relative absence of wake-promoting circadian signal.

Though the original two-process model, stating that process S and C interact together to determine the main features of sleep regulation, remains the prevalent theoretical model, it has been re-evaluated since, to account for more recent empirical advances (Borbély et al., 2016). Among these aspects is the fact that, unlike in the original model where process S and C were thought to interact at critical moments (i.e. sleep onset and wake up time), the reappraisal states that the two processes interact continuously and influence each other, such that the expression of one is always affected and modulated by the second process. For example, forced desynchrony protocols, in which experimental participants live in a 20h or 28h sleep-wake cycle to which the circadian clock cannot adapt and therefore “free-run” with a cycle of about ~24h decoupled from sleep-wake behaviour, have shown that circadian amplitude is lower when sleep pressure is high, and conversely is high when sleep pressure is low (Dijk & Czeisler, 1995), while in turn, SWA is partly determined by the circadian phase in which the wake extension occurs (Vyazovskiy, Achermann, & Tobler, 2007).

Brain networks underlying sleep-wake regulation

For a long time, sleep was thought to be an inactive state, a sort of opposite of wakefulness similar to unplugging a cable. In that sense, the idea that sleep is the fruit of the coordination of several specific brain regions, different from the ones responsible for wakefulness is relatively recent. It has been first put forward by Von Economo, a Viennese neurologist that worked during an epidemic of encephalitis lethargica, around the beginning of last century, but the idea did not meet a large echo at the time. He noticed patients with midbrain and posterior hypothalamus lesions had troubles staying awake, and were sleeping most of the time, while other patients with lesions of the preoptic area and basal forebrain were displaying severe insomnia (Saper, Chou, & Scammell, 2001; Scammell et al., 2017; Von Economo, 1930). Von Economo thus concluded that anterior regions of the hypothalamus were part of a sleep-promoting network, while posterior hypothalamus constituted a wake-promoting region. Since then, our understanding of the neural circuitry underlying sleep and wakefulness regulation has evolved. In this section, we will review the current knowledge on the matter, and see how the mutual inhibition between sleep- and wake-promoting regions constitutes what has been dubbed a “flip-flop switch” that allows swift transitions between sleep and wakefulness and prevent intermediate states (Saper & Fuller, 2017; Saper, Fuller, Pedersen, Lu, & Scammell, 2010).

Wakefulness

In the late forties, Moruzzi and Magoun showed that stimulation of the reticular formation with electrical current in anesthetised cats leads to EEG activity reminiscent of wakefulness (Scammell et al., 2017). This led to the long prevailing concept of arousal being in great part controlled by the ascending reticular activating system (ARAS), though it was shown only later that not the whole reticular formation is responsible for maintaining wakefulness. Moreover, the wake-promoting pathway originating from the ARAS divides into 2 branches, one track innervating the cortex via the thalamus, the other via the hypothalamus (Saper et al., 2001). The pathway innervating the thalamus (dorsal pathway) originates mainly from the cholinergic pedunculo pontine and laterodorsal tegmental nuclei (PPT-LDT) (Morrell, Palange, Levy, & De Backer, 2012; Saper et al., 2001; Saper, Scammell, & Lu, 2005) (see **figure 8**). PPT-LDT neurons fire rapidly during wakefulness as well as REM sleep (see section on REM sleep), which is why they have been suggested to play a role in cortical activation (Saper et al., 2001, 2010). The hypothalamic pathway (ventral pathway), on the other hand,

takes its roots in the caudal midbrain and rostral pons (Saper et al., 2001) including the locus coeruleus (LC), main source of noradrenaline, and serotonergic dorsal and median raphe nuclei and parabrachial nucleus, as well as dopaminergic neurons contiguous to the dorsal raphe nucleus (Morrell et al., 2012; Saper et al., 2010). Interestingly, neurons from those cell groups are active during wakefulness, while they exhibit decreased activity during NREM, and are silent during REM sleep (Saper et al., 2010).

Activation of the cortex is also driven by orexinergic neurons from the hypothalamus, and cholinergic neurons in the basal forebrain (Morrell et al., 2012). Indeed, orexinergic neurons from the lateral hypothalamus project heavily to the LC, from which the noradrenergic projections favours the cortical arousal of wakefulness (Pace-Schott & Hobson, 2002). Additionally, glutamatergic neuron from the rostral pons situated in the parabrachial nucleus and neighbouring precoeruleus area seem to be involved in wakefulness, through projections to the lateral hypothalamus, basal forebrain and to the cortex (Saper & Fuller, 2017).

NREM sleep

Initially, NREM sleep was believed to appear following the interruption of subcortical excitatory inputs to the cortex, however, more recent work allowed to delineate several NREM promoting subcortical regions, essential for producing NREM, most of them GABAergic (Gompf & Anaclet, 2020). The ventrolateral preoptic area (VLPO), situated in the anterior hypothalamus, contains GABAergic/galalinergic neurons that inhibit wake-promoting areas (Morrell et al., 2012). The parafacial zone, located in the brainstem, contains GABAergic neurons which are both needed and sufficient to cause SWS. The ventral tegmental area (VTA), which at first was recognised as a wake-promoting region via its dopaminergic projections, was later found to play both a wake-promoting role via its ventral striatum projection, as well as a sleep promoting role through its dorsal projection (Gompf & Anaclet, 2020). PPT-LDT neurons, very active during wakefulness, are mostly silent during NREM sleep.

REM sleep

The main region involved in REM control is the brainstem, where both REM-on and REM-off neurons are located (Gompf & Anaclet, 2020; Lu, Sherman, Devor, & Saper, 2006), with the sublaterodorsal nucleus (SLD) containing glutamatergic REM-on neurons. SLD neurons form two groups: one group with a rostral projection to the forebrain, participating in

cortical activation and hippocampal theta activity, while the other is involved in muscle atonia via caudal projections to the ventromedial medulla and spinal motoneurons (Gompf & Anaclet, 2020). During REM sleep, PPT-LDT neurons fire rapidly, as during wakefulness. REM-off neurons are composed of: GABAergic neurons in the ventrolateral periaqueductal gray (vlPAG) and lateropontine tegmentum; acetylcholinergic PPT/LDT neurons; noradrenergic locus coeruleus (LC) neurons; and serotonergic dorsal raphe (DR) neurons). While SLD GABAergic neurons inhibit REM-off neurons, those, in turn, inhibit REM-on neurons.

EEG activation is thought to be the result of an increase in firing rates of reticular, thalamocortical and cortical neurons (Pace-Schott & Hobson, 2002). Rapid eye movements are caused by the phasic firing of reticular and vestibular neurons (Pace-Schott & Hobson, 2002). Crucially important muscle atonia, presumably preventing the enactment of dreams during REM sleep, seems to originate from neural circuits in the pons (Scammell et al 2017). The caudal edge of the pons and the sublateralodorsal nucleus (SLD) or subcoeruleus appear to be essential to produce atonia, possibly through activation of the ventromedial medulla (VMM) which would in turn inhibit motor neurons in the spinal cord, or by directly activating spinal interneurons which would then inhibit motor neurons, or possibly via both (Lu et al., 2006; Scammell et al., 2017).

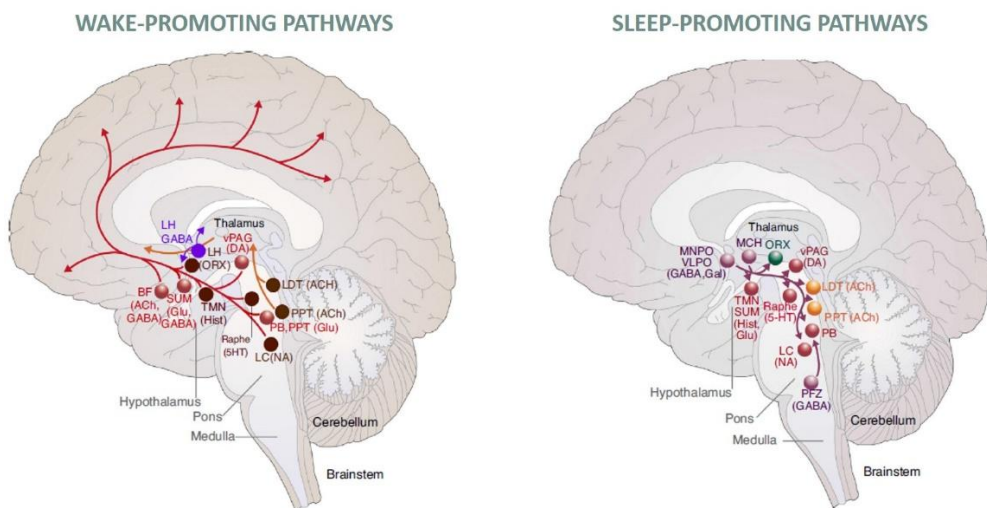


Figure 8. Schematic representation of the main neural circuits underlying wake (left) and sleep (right). Main wake-promoting inputs take their roots in glutamatergic (Glu) neurons from the brainstem projecting to the basal forebrain (BF), and cholinergic (ACh) and GABAergic neurons in BF to the cortex. GABAergic neurons from the lateral hypothalamus (LH) inhibit sleep-promoting regions in

the thalamus and preoptic area. Sleep-promoting pathways consist mainly in GABAergic neurons in the ventrolateral preoptic (VLPO) area and median preoptic (MnPO) inhibiting most of the wake-promoting system. REM is produced by the activity of glutamatergic neurons in the subcoeruleus area and cholinergic neurons from the laterodorsal and pedunculopontine tegmental nuclei (PPT). Adapted with permission from (Saper & Fuller, 2017).

Conclusion

The flip-flop switch models a mutually inhibitory, bistable feedback loop that, similarly to an electrical switch, creates two types of states (sleep/wakefulness) and tends to avoid intermediate states (Adamantidis, Gutierrez Herrera, & Gent, 2019; Saper et al., 2001, 2010). In this model, a specific role for the orexinergic system has been put forward. Orexinergic projections from the lateral hypothalamus to crucial wake-promoting regions (mainly the LC) have been proposed to play a stabilising role for wakefulness, preventing ill-timed transitions to sleep (Pace-Schott & Hobson, 2002; Saper et al., 2001). A great example of perturbations in that process can be found in narcoleptic patients, where a substantial reduction in the lateral hypothalamus orexinergic neurons can be observed, resulting in a higher probability of abnormal wake-to-sleep transitions (Pace-Schott & Hobson, 2002). Sleep is engaged by projections from the anterior hypothalamus, containing sleep-active neurons, which in turn inhibit wake-active regions of the hypothalamus, basal forebrain and brainstem. A specific brainstem switch containing REM-on and REM-off neurons was proposed to be responsible for NREM and REM transitions (Gompf & Anaclet, 2020; Lu et al., 2006).

The original flip-flop switch model provides crucial insights into the sleep-wake brain circuitry. Meanwhile, the involvement of other neural populations via novel and powerful molecular genetic tools is still being unveiled (Gompf & Anaclet, 2020). Also, as will be described in the next section, the idea of a clear-cut transition between different states is no longer the prevailing viewpoint, as it is now well established that different substates can coexist in the brain.

Global versus local sleep

For many years, it was accepted that sleep constitutes a global phenomenon, and affects the brain uniformly. As such, sleep and wakefulness are often considered as mutually exclusive states. Though they do have distinct behavioural and EEG characteristics, it has been shown that some of the features of sleep can be found, at a local level, during wakefulness and vice versa (Krueger, Nguyen, Dykstra-Aiello, & Taishi, 2019; Siclari & Tononi, 2017). As such, various degrees of sleep and wakefulness can co-occur in different regions of the brain. A spectacular example of this is the fact that some species of marine mammals, like dolphins or whales, show unihemispheric sleep, with half of their brain sleeping, while the other half is showing all EEG signs of wakefulness (Mukhametov, 1985; Sekiguchi, Arai, & Kohshima, 2006). In humans, a study reported that during the first night in a laboratory, the left hemisphere shows less SWA and has a lower stimulation threshold than the right one, possibly as a way of monitoring the potential threats of a novel environment (Tamaki, Bang, Watanabe, & Sasaki, 2016). Local aspects of sleep can also be modulated by prior wakefulness content (Rector, Schei, Van Dongen, Belenky, & Krueger, 2009). It was shown that regions intensely recruited during wakefulness show increased local SWA during subsequent sleep (Huber, Ghilardi, Massimini, & Tononi, 2004). The SHY (see section on functions of sleep) relates this change in SWA to synaptic downscaling (Tononi & Cirelli, 2003). Conversely, a reduction of activity over certain brain areas, such as the sensorimotor cortex when immobilising a limb for a period of 12h, leads to a local decrease of SWA (Huber et al., 2006). In a wake-extension protocol, a local increase in theta activity was shown to be task-dependent (Hung et al., 2013). Recently, a study showed local modulation not only of homeostatic process, but of brain circadian rhythmicity (Muto et al., 2016). While most cortical areas seem to be influenced by homeostatic regulation, many subcortical responses show strong circadian modulation (ibidem).

Interestingly, the local modulation of sleep-wake states can be observed in transitions from sleep to wakefulness, or conversely. While falling asleep, anterior regions display SWA typically earlier than posterior regions (Marzano et al., 2013). More surprisingly perhaps, intracortical recordings showed that the hippocampus displays spindles minutes before the cortex sleeps; this mechanism might be responsible for the frequent amnesia related to the period right before falling asleep (Sarasso et al., 2014). Conversely, the waking up process sees blood flow restored in neocortical regions lastly (Balkin et al., 2002), and increased SWA in posterior regions relative to pre-sleep wakefulness, which most likely is related to sleep

inertia (Marzano, Ferrara, Moroni, & De Gennaro, 2011), a temporary state of lowered arousal right after awakening which is characterised by a transiently lowered performance (Tassi & Muzet, 2000). Also, a most striking example of local sleep regulation is seen in some sleep pathologies, such as sleep walking, where wake and sleep features seem to coexist in different regions of the brain (Terzaghi et al., 2012).

It is thus now well recognised that sleep and wakefulness not only represent two extremes of a continuum that can coexist to some extent in the brain, but those two states exert a mutual influence on each other. While the co-occurrence of sleep- and wake-like states in different brain regions can be a marker of sleep-related pathologies, in the healthy subject, sleep is regulated locally and influenced by various factors such as prior experiences and learning.

Genetic regulation of sleep

A growing body of research has been concentrating on elucidating the contribution of genes in the regulation of the sleep-wake cycle. Several aspects of sleep and circadian rhythmicity are highly constant within individuals, while showing relatively high inter-individual variability. Understanding the genetic basis of these features seems like a promising way of expanding our understanding of sleep, whether with regards to its function, its neurobiology or the mechanisms underlying its pathologies. Nevertheless, studying the role genetics play in human sleep regulation constitutes a challenge, mainly because sleep is a complex phenotype which is undoubtedly impacted by several confounding factors such as environmental influences, which render genetic factors difficult to unravel, but also because of obvious methodological limitations related to the brain inaccessibility which unable genetic and molecular *in vivo* studies (Hor & Tafti, 2017).

The study of twins (monozygotic versus dizygotic), as well as population- and family-based investigations have shown that around 50% of diurnal preference (that is, the timing of nocturnal sleep and of daytime alertness variations, also called morningness and eveningness types (Hor & Tafti, 2017; Horne & Ostberg, 1976)) and 20-25% of habitual bedtime are, in fact, inheritable (Gottlieb, O'Connor, & Wilk, 2007; Landolt & Dijk, 2017). The sleep features showing the highest inheritability (up to 98%) is the EEG (Hor & Tafti, 2017). Indeed, both NREM sleep (Ambrosius et al., 2008) and wake EEG (Kondacs & Szabo, 1999) have been reported to have strong genetic underpinnings, to the point that EEG features, termed as the EEG fingerprint, have been proved to be stable over time and experimental conditions (De Gennaro et al., 2008; De Gennaro, Ferrara, Vecchio, Curcio, & Bertini, 2005). Several aspects of sleep, such as the amount of slow wave sleep (Linkowski, 1999; Linkowski, Kerkhofs, Hauspie, & Mendlewicz, 1991), but also spindle density (Purcell et al., 2017) could have strongly contributing genetic factors. Investigations on REM sleep, on the other hand, seem to yield mixed results regarding its potential genetic influence (Adamczyk et al., 2015; H. P. Landolt, 2011; Linkowski et al., 1991).

Candidate genes related to circadian rhythmicity have been relatively well studied, as several genes such as the CLOCK gene, playing a role in diurnal preference (Katzenberg et al., 1998) and sleep duration (Allebrandt et al., 2010), and Period2 and 3 (Per2/Per3), associated with sleep phase advance (Toh et al., 2001), and diurnal preference and phase delay (Archer et al., 2010, 2003), respectively, have been identified. Investigations regarding the

genetic underpinnings of sleep homeostasis are, however, far more in their infancy (Allada, Cirelli, & Sehgal, 2017; Donlea, Alam, & Szymusiak, 2017).

It is important to underline that sleep and circadian rhythmicity are complex phenotypes, with different features being likely governed by numerous genes. Those, in turn, do not operate in isolation but influence and are in influenced in turn by the environment as well as factors such as age. Untangling the relationships between genes and sleep is will certainly keep scientists busy for some time, and investigation of very large datasets encompassing objective sleep data (EEG/actimetry) will undoubtedly play a key role in this regard.

The functions of sleep

Perhaps surprisingly, the question of the function(s) of sleep remains an open one. Though many hypotheses have been proposed over the years, none has reached unanimity or successfully untangled all sleep-related phenomena. In this section, we will make a brief overview of the various theories that have been proposed on the subject.

Metabolism regulation

We all experienced at some point the increased craving for high calorie food after a sleepless night. Well it is not without reason. Research has shown that sleep restriction leads to a decrease of leptin, a satiety hormone, and an increase in ghrelin, an appetite hormone (Morselli et al., 2010). Importantly, the increase in food intake resulting from a prolonged exposition to sleep restriction exceeds the increase of energy expenditure (Markwald et al., 2013). Sleep restriction is linked to increased risk of developing type II diabetes (Morselli, Guyon, & Spiegel, 2012; Morselli, Leproult, Balbo, & Spiegel, 2010; Peigneux & Leproult, 2014). In clinical populations, obstructive sleep apnoea (OSA) has been linked to insulin resistance (Ip et al., 2002). A very high prevalence of OSA was found in type II diabetes patients (from 58 to 86%), while the percentage of diabetic patients is also higher in OSA patients compared to healthy controls (Morselli et al., 2012). Interestingly, specific SWS suppression through auditory stimuli, while preserving REM sleep and total sleep time, also led to reduced glucose tolerance and greater diabetes risk (Herzog et al., 2013). Together, these results show that sleep is involved in several metabolic and hormonal regulatory processes, which are impacted when one messes with sleep quality/quantity. Additionally, sleep has been postulated to hold an energy conservation purpose (Scharf, Naidoo, Zimmerman, & Pack, 2008), while it has been shown that brain energy metabolism is only slightly reduced (~15-20%) during sleep, implying that sleep does not serve solely energy conservation purposes (Madsen et al., 1991, from Jessen, Munk, Lundgaard, & Nedergaard, 2015).

Memory consolidation and brain plasticity

It is now well established that sleep plays a crucial, active role for memory consolidation (Diekelmann & Born, 2010; Klinzing, Niethard, & Born, 2019; Maquet, 2001; B. Rasch & Born, 2013; Stickgold, 2005; Stickgold & Walker, 2005). Particularly, the contribution of NREM sleep (with a special role for spindles and SW) for declarative memory and of REM sleep (and sharp wave ripples) for procedural learning, have been put forward

(Maquet et al., 2010). Two important models have been proposed to account for the role that sleep plays in memory consolidation, that is the temporal process through which an initially fragile memory trace is strengthened into long-term memory systems (Peigneux & Leproult, 2014). The first model, or hippocampo-neocortical dialogue model (Buzsáki, 1996; Rasch, Born, & Gais, 2006), proposes that memory traces are first acquired and temporarily stored in the hippocampus, before being moved to neocortical areas for long-term storage. This transfer from hippocampal areas to the neocortex was proposed to take place at least in part during sleep (Klinzing et al., 2019). On the other hand, the synaptic hypothesis theory (Tononi & Cirelli, 2006) suggests that, when learning a novel information, a local increase in synaptic potentiation takes place, and an increase in energy and space costs in the locally involved neuronal populations can be observed. This in turn leads to a synaptic saturation and reduced plasticity. Subsequent sleep, particularly SWS, offers the opportunity to downscale synaptic strength back to baseline levels, as well as restore synaptic plasticity, all the while keeping a trace of the potentiated synapses related to this novel learning. Learning also results in proportionally and locally increased slow oscillations (see section on global vs local sleep), which are pointed as the perfect occasion to refine synaptic weight and improve signal-to-noise ratio by enhancing relevant memory traces and cleaning or removing the weaker/less relevant ones. Though the SHY hypothesis has many supporting arguments, not all available evidence seems to support this theory (Frank, 2012, 2013).

Brain metabolites clearance

More recently, research has been pointing towards a role of sleep in brain metabolite clearance. Indeed, it was found that in mice, amyloid-beta levels in the interstitial (ISF) fluid are tightly linked to the sleep wake-cycle, and are increased following sleep deprivation (Kang et al., 2009). Also, the discovery of the glymphatic system, a process of brain metabolite clearance promoted by sleep (Jessen et al., 2015; Plog & Nedergaard, 2018) constitutes a key finding. It was further found that the volume of the interstitial space is increased by 60% during sleep, facilitating the elimination of brain metabolites by convective flow (Xie et al., 2013). This part will be developed in more details in the section about sleep and Alzheimer's disease.

Mental health

Insomnia and sleep problems in childhood have been pointed out as predictive of future development of depression and anxious/depressive symptom, while not the opposite, suggesting a causal link between sleep disturbances and depression (Wiebe, Cassoff, &

Gruber, 2012). Shorter REM latency, increased REM duration and density, increased SOL and reduced SWA are related to higher risk for depression (ibidem). Though the links are not entirely understood, they most probably involve serotonergic response, as well as the prefrontal cortex (PFC) (Peigneux & Leproult, 2014). Decreased response to serotonin was shown following sleep restriction, thus impairing the brain's ability to optimally use available serotonin (Wiebe et al., 2012). Regarding the PFC, its role has been put forward in depression, while lesions in some parts of the PFC in rats have been shown to produce an increase in REM sleep and a decrease in REM latency, similarly to what is observed in major depression, suggesting a critical role of some the PFC for the modulation of depressive and sleep behaviours (Chang, Chen, Qiu, & Lu, 2014). Interestingly, an antidepressant effect of sleep deprivation (known to markedly impact frontal regions) in depressive patients has also been reported (Borbely & Wirz-Justice, 1982).

Sleep and ageing

Sleep undergoes a series of changes throughout the lifetime, which are now quite well documented (Gaudreau, Carrier, & Montplaisir, 2001; Junxin Li, Vitiello, & Gooneratne, 2018; Mander, Winer, & Walker, 2017). From the first months of life to old age, sleep is modified with respect to both its regulating processes (Lopez, Barateau, & Dauvilliers, 2019; Ohayon, Carskadon, Guilleminault, & Vitiello, 2004). From birth to infancy, sleeping periods change from being scattered around the clock in naps (polyphasic sleep) to progressively constituting one consolidated period of night-time sleep (Shimko, 2019). With maturational changes, babies' sleep evolves from quiet/active (and indeterminate) sleep, before 2 months of age, to the emergence of proper NREM/REM sleep (Barry, 2021). The amount of daily sleep is also drastically reduced from the neonatal period (~17h of sleep per 24h), to an amount closer to that of adulthood around late adolescence (Barry, 2021; Ohayon et al., 2004).

Globally, from infancy to late adulthood, several aspects of sleep architecture were reported to change. Total sleep time, sleep efficiency, as well as SWS were all shown to gradually decrease, while WASO reportedly increases with age (Carrier et al., 2011; Gaudreau, Carrier, et al., 2001; Ohayon et al., 2004) (see **figure 9**).

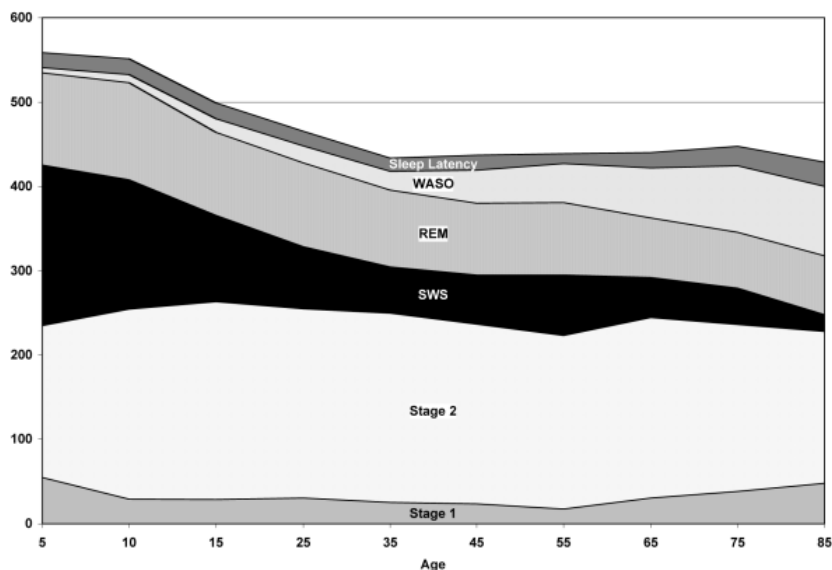


Figure 9. Illustration of the general mean time spent in each sleep stage (N1/N2/N3/REM), as well as wake after sleep onset (WASO), sleep latency and total sleep time (in minutes) across the lifespan. Reproduced with permission from (Ohayon et al., 2004)

Changes in sleep, starting as early as the fifth decade of life, are part of the normal ageing process (Carrier et al., 2011; Mander et al., 2017). At the rough, architectural level, ageing is associated with a lower ability to initiate sleep (Mander et al., 2017), as reflected by a longer sleep onset latency. Overall, a shorter total sleep time and sleep efficiency is observed, as well as an increase in time spent in N1 and N2 and a reduction of time spent in N3 (Březinová, 1975; Ohayon et al., 2004; Redline et al., 2004). The ability to maintain sleep is also impacted by ageing, resulting in more frequent/longer awakenings, as observed by increased WASO (Junxin Li et al., 2018; Miner & Kryger, 2017).

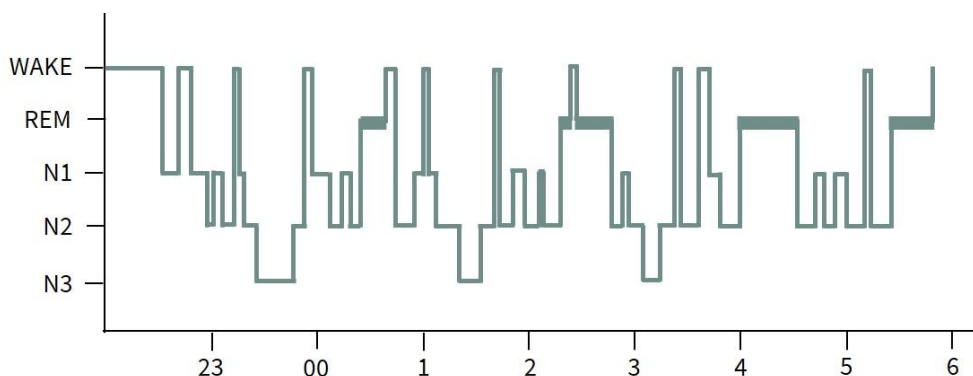


Figure 10. A sleep hypnogram typical of older individuals, characterised by shorter time spent in N3, more frequent awakenings during the night, and an earlier bed and wake-up time

At the finer level, a drop of general EEG power and amplitude has been reported, predominantly observable over the delta band in anterior derivations (Gaudreau, Carrier, et al., 2001; Landolt & Borbély, 2001). SWA is generally described as reduced (Carrier et al., 2011), while the SWA increase in response to a sleep deprivation is also less pronounced (Gaudreau, Morettini, Lavoie, & Carrier, 2001). The finer modifications observable at the level of sleep microstructure will be discussed in the chapter describing the key graphoelements of the sleep EEG.

Though ageing is also associated with an increased prevalence of sleep related disorders (such as sleep apnoea, periodic limb movements during sleep etc), studies on individuals devoid of such show a similar pattern of declining sleep quantity and quality, suggesting that, to some extent, the ageing process as such gives rise to such changes (Ohayon et al., 2004).

Ageing and circadian/homeostatic influences

Circadian processes appear to be modified in ageing. Reportedly, the timing and intensity of circadian signals seem to be impacted: older individuals exhibit earlier bed- and wake-up times than their younger counterparts, in fact, this slight phase advance would appear to be of ~1h (Dijk, Duffy, & Czeisler, 2000). The strength of circadian signalling also appears to be reduced, as reflected by lower melatonin secretion patterns, a lower circadian modulation of REM sleep, as well as higher sleepiness levels during the wake-maintenance zone (Cajochen, Münch, Knoblauch, Blatter, & Wirz-Justice, 2006; Münch et al., 2005). Dampening of cortical excitability dynamics have also been proposed to reflect age impact on both circadian and homeostatic processes (Gaggioni et al., 2019).

Bouts of wakefulness during the night are more common, and, conversely, intrusion of sleeping periods during the day (napping) frequently occur (Bliwise, Ansari, Straight, & Parker, 2005; Park et al., 2001). Sensitivity to a circadian challenge, such as sleep deprivation, is reduced, with smaller cognitive impairment under sleep deprivation, explained by a reduction of circadian signalling and homeostatic build-up of sleep need (Taillard, Gronfier, Bioulac, Philip, & Sagaspe, 2021). In jet-lag situations, though the speed of adjustment of core body temperature rhythms to the new time is reportedly not different in older individuals compared to young, they have larger increase of time spent awake and earlier wake-up times than their young counterparts (Moline et al., 1992). Importantly, deteriorations in the SCN in *post-mortem* studies have been reported alongside the reduced melatonin secretion (Hofman & Swaab, 2006). It appears therefore that circadian signalling, whether with respect to its intensity or timing, is impacted in ageing.

Though some reports have concluded to a lack of change in homeostatic process in ageing (Campbell & Feinberg, 2005), most reports describe a reduction in SWA, which is considered to arise from an impacted build-up of sleep need during wakefulness (Cajochen et al., 2006; Gaudreau, Morettini, et al., 2001; Münch et al., 2004).

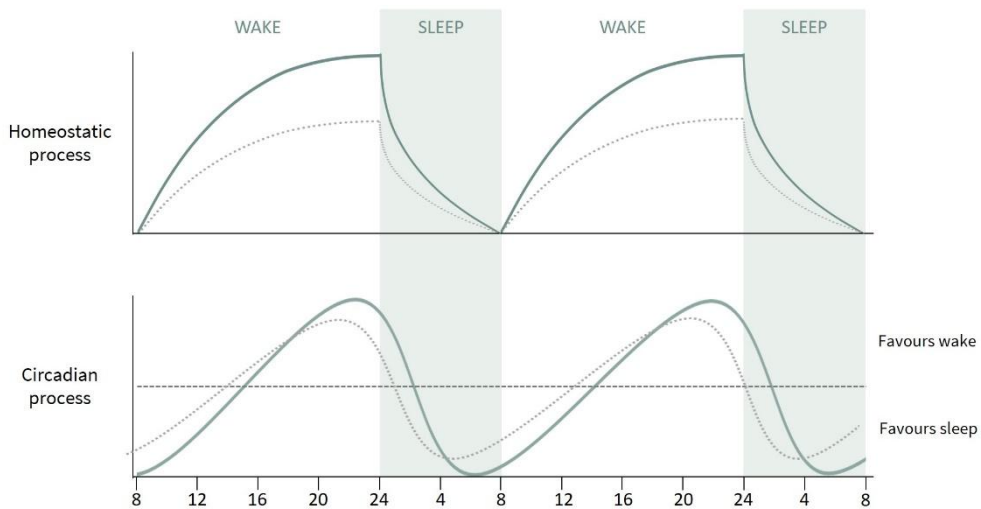


Figure 11. Illustration of the modifications in the homeostatic (upper panel) and circadian (lower panel) processes in adulthood and in ageing. The dark line represents the intact processes observed in a adulthood, while the dashed grey line represents the alterations observed in ageing.

To conclude, the ageing process markedly changes sleep, with regards to its architecture, but also to its finer microstructural level, as will be developed in the next chapter. It has to be underlined that circadian and homeostatic processes are challenging to disentangle, and the changes that seem to occur in ageing are hard to attribute to one of them, or their interaction (Munch, Knoblauch, Blatter, Wirz-Justice, & Cajochen, 2007). Additionally, it is difficult to attribute changes in circadian response solely to modifications in circadian signalling, to alterations of the SCN or other parameters affecting circadian timing such as zeitgebers, which are inarguably less clearly pronounced in ageing. Indeed, older individuals are in a different context than mostly studied younger adults, as after retirement, professional and social context is less present. Similarly, reported diminution in SWA is certainly affected as well by cortical thinning, and the latter even mediates the effect of age on SW generation (Dubé et al., 2015).

Chapter II: Sleep microstructure

Undoubtedly, sleep is not constituted of static bits of homogeneous activity, but represents a more dynamic process with rapidly evolving EEG components. In this chapter, we will describe the main finer gradient events observable during human sleep at the surface EEG, that is, the microstructure composing sleep. In this chapter, we will explore vertex sharp waves, spindles, K-complexes, slow waves, REM-alpha bursts, sawtooth waves, arousals and cycling alternating patterns (CAP). For each element, we will overview their description and detection, the current knowledge of their underlying mechanisms, the factors known to influence them and their functional correlates.

Vertex sharp waves



Description & detection

Vertex sharp waves (VSW), also called vertex sharp transients or V waves, are well delineated waves with a duration of less than 500ms, which are seen in late stage N1 and early stage N2, quite often at the onset of sleep, and are most prominent over central derivations (Iber et al., 2007; Keenan & Hirshkowitz, 2011; Rodenbeck et al., 2006). They are mono- or biphasic waves, often constituted of a small positive peak preceding a larger negative one, with amplitude sometimes as large as 200 μ V (Rodenbeck et al., 2006). They have been termed as the first EEG feature that is unique to sleep (Stern et al., 2011).

Mechanisms

Despite their role that might be crucial for the process of falling asleep, little is known about their brain correlates and functional significance (Rodenbeck et al., 2006). A study using both EEG and fMRI reported significant increase in blood oxygen level dependent (BOLD) signal in medial central, lateral precentral, posterior superior temporal and medial occipital cortices concomitant with VSW occurrence on the EEG (Stern et al., 2011). The authors argue the paracentral region is likely to be the VSW generator as it is immediately underlying the

region of maximum V waves voltage, while the other associated regions could point towards a role of V waves in sensory (ibidem).

Influencing factors

The frequency of VSW is reportedly increased after sleep deprivation, suggesting a relationship with sleep homeostasis (Peszka & Harsh, 2002).

Impact of ageing

The characteristics of VSW across the lifespan remain understudied. One study reported a decrease in the number and amplitude of VSW with age (Kim, Lee, Han, & Jung, 2012).

Functional correlates

VSW have been shown to appear spontaneously, but also as a response to external stimulus (Rodenbeck et al., 2006; Sekine, Niiyama, Kutsuzawa, & Shimizu, 2001). These characteristics earned them a hypothetical role of sleep maintenance or response to external stimulation, similarly to K-complexes (see section on K-complexes) (Bastien, Crowley, & Colrain, 2002).

Spindles



Description & detection

Spindles are brain waves detected on the scalp EEG in the 11-16 Hz frequency range, which have a very specific waxing-and-waning shape, characterised by a progressively increasing then decreasing amplitude, and a duration of more than 500ms (Iber et al., 2007), typically between 1 and 3s (Mircea Steriade, McCormick, & Sejnowski, 1993). These oscillations usually peak around 100 μ V of amplitude, and persist for ~6-15 cycles (Adamantidis et al., 2019). Sleep spindles can be subdivided into two types, related to their main frequency (fast and slow spindles) (De Gennaro & Ferrara, 2003). Slow spindles (usually around 12Hz), more prominent over frontal regions, gradually decrease over the course of a night while fast spindles (~14Hz) are more visible over central and parietal derivations (Werth,

Achermann, Dijk, & Borbély, 1997). Most spindles show a progressive decrease in internal frequency from their beginning to their end (Schönwald, Carvalho, Dellagustin, de Santa-Helena, & Gerhardt, 2011; Zerouali et al., 2014).

Mechanisms

Spindles are generated in the thalamus as a result of synaptic interplay between the thalamic reticular nucleus (TRN) inhibitory neurons and thalamocortical excitatory neurons, and their interaction with pyramidal neurons of the cortex (Adamantidis et al., 2019; Sejnowski & Destexhe, 2000; Mircea Steriade et al., 1993). Some observations speak in favour of a primary brainstem contribution to thalamic and corticothalamic spindles generation, as changes in excitability of the brainstem have been found to precede spindle occurrence (Kohsaka, Sakai, Kohsaka, Fukuda, & Kobayashi, 2000). A study combining MRI with EEG showed that slow spindles are associated with an increase in cortical activity in the superior frontal gyrus, while fast spindles involve the activity of the mesial frontal cortex, the hippocampus, as well as cortical regions implicated in sensorimotor processing (Schabus et al., 2007). In mice, optical stimulation of cholinergic neurons in the basal forebrain and brainstem, projecting to the TRN was found to induce spindles (Ni et al., 2016). Interestingly, early synchrony during spindles is associated with intra-hemispheric connectivity, while late synchrony with higher inter-hemispheric connectivity (Zerouali et al., 2014). K-complexes (see below) and spindles have been reported as strongly related, as the first has been described as playing an important role in triggering the latter (Amzica & Steriade, 2002). Recently, spindles have also been shown to occur locally, particularly in the thalamus, where they exhibit lower spectral power relative to spindles found in multiple neocortical regions (Bastuji, Lamouroux, Villalba, Magnin, & Garcia-Larrea, 2020)

Influencing factors

Several factors have been found to negatively influence spindles density, psychiatric diseases (Astori, Wimmer, & Lüthi, 2013), or the intake of certain medication such as benzodiazepine (Kubicki, Haag- Wüsthof, Röhmel, Herrmann, & Scheuler, 1988). Spindles have been shown to be reduced following sleep deprivation, suggesting an inverse homeostatic relationship between spindles and slow waves (Knoblauch, Martens, Wirz-Justice, & Cajochen, 2003). A circadian modulation of spindles frequency and density has been reported, as spindle density has been described as increased and intra-spindle frequency as reduced in

melatonin secretion phase, with a parietal dominance (Knoblauch, Martens, Wirz-Justice, Kräuchi, & Cajochen, 2003; Knoblauch et al., 2005).

Impact of ageing

Age has been reported to negatively impact spindles, whether their density, amplitude or duration (Crowley, Trinder, Kim, Carrington, & Colrain, 2002; Nicolas, Petit, Rompré, & Montplaisir, 2001; Rudzik et al., 2018). An age-related impact on the circadian modulation of spindles frequency has been reported, as said modulation was reported as attenuated and occurring at an earlier phase in older individuals compared to young (Knoblauch et al., 2005).

Functional correlates

Importantly, spindles have been found to play a crucial role in cognition, as they have been particularly linked to memory performance (Ulrich, 2016). Indeed, spindle density was found to be related to memory formation for verbal learning - such as word-association or word-pair learning tasks (Gais, Mölle, Helms, & Born, 2002; Schmidt et al., 2006), integration of novel words to the vocabulary (Tamminen, Payne, Stickgold, Wamsley, & Gaskell, 2010) – and visuospatial memory (Clemens, Fabo, & Halasz, 2006). Spindle activity has also been related to procedural learning (A. Morin et al., 2008). Interestingly, the active part played by spindles in memory formation is supported by studies inducing spindle activity increments, whether by medication (Mednick et al., 2013; B. Rasch, Pommer, Diekelmann, & Born, 2009) or transcranial alternating current stimulation (Marshall, Helgadottir, Mölle, & Born, 2006) which all led to improved memory performance. Crucially, this increase in spindle activity was reported to occur in task-related brain regions (Nishida & Walker, 2007). A sleep protective role for spindles has also been hypothesised, though a study on night-time transportation noise exposure reporting a lack of difference in sleep continuity and structure in individuals with higher and lower spindle rates tends to suggest they may rather be a trait phenomenon than a sleep-protective factor (Rudzik et al., 2018). An fMRI/EEG study on acoustic tone processing during wakefulness and NREM sleep showed a reduction in the transmission of information during spindles (Dang-Vu et al., 2011), suggesting spindles are associated to a decrease in the transmission of external stimuli, rather than its elimination.

K-complexes



Description & detection

A K-complex (KC) is defined on an EEG trace as a sharp, large amplitude, biphasic wave of more than 0.5 sec duration. It was first observed by Loomis and colleagues (Loomis, Newton, & Hobart, 1938), and its shape, evocative of the letter K might be where it got its name from (Buzsáki, 2009), or it might be taken from the first letter of the word knock (as it was observed after knocking on the door of the sleeping subject) (Adamantidis et al., 2019). It is a waveform seen in stages N2 and N3. Similarly to the rest of slow waves found in sleep, they are predominantly found on frontal derivations (Halász, 2016).

KC comprise three components: a short positive one around 200ms (P200), a longer negative deflection around 550ms (N550), and a large positive peak at 900ms (P900) (Gandhi & Emmady, 2020; Halász, 2016). In the first descriptions of the KC, the Loomis group depicted K-complexes as being composed of a slow frequency component, and a superimposed faster component, in the frequency range around 8 or 14Hz (Davis, Davis, Loomis, Harvey, & Hobart, 1939). It appears that these faster frequencies were in fact two other waveforms, that is most likely alpha bursts and spindles, respectively. The important observation that KC would often, but not always, be followed by an arousal, was already made very early (Roth, Shaw, & Green, 1956).

Mechanisms

The origin of KC seem to be cortical and subcortical, with particularly a thalamic component (Jurko & Andy, 1978) . The initial positive deflection reflects cortical neurons excitatory state, while the following negative echoes neuronal hyperpolarisation (Amzica & Steriade, 2002). With sleep deepening, KC exhibit a small increase in their main frequency, which might be explained by the progressive removal of the excitatory drive from thalamocortical neurons, enabling higher hyperpolarisation levels of cortical neurons' membrane potentials (ibidem).

Influencing factors

Benzodiazepine administration was reported to decrease K-complex density (Jobert, Poiseau, Jähnig, Schulz, & Kubicki, 1992; Kubicki et al., 1988). Studies in alcoholic subjects show that K-complex density as well as the amplitude of the negative deflection are both reduced (Colrain, Nicholas, & Baker, 2014).

Impact of ageing

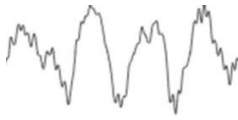
The density of K-complexes has been reported to decrease with age (Crowley et al., 2002). Also, a study investigating spontaneous K-complexes in healthy older and AD patients reported that a substantial decrease (>40%) was observed in AD patients versus control, which allowed a correct classification of 80% (De Gennaro et al., 2017).

Functional correlates

K-complexes have a double character, as they can be seen in regular, undisturbed sleep, but can also be triggered in any NREM sleep stage (Colrain, 2005) by stimulation through any sensory modality (Halász, 2005, 2016). They can be evoked by a stimuli (auditory, tactile or somatosensory), but also by non-modality-dependent stimulation, such as an increase in respiratory effort (Adamantidis et al., 2019; Webster & Colrain, 1998). Evoked and spontaneous K-complexes globally share the same EEG characteristics (Adamantidis et al., 2019).

The functions of KC have long been a matter of debate. It has been put forward they might represent a form of arousal response, or serve as sleep protective role, inductive of SWS. In favour of the latter are some interesting facts: K-complexes density (whether spontaneous or elicited) are increased prior to transition towards SWS compared to transition towards REM sleep; evoked K-complexes are more likely to be seen in N2 than N3; and the prevalence of spontaneous K-complexes decreases over consecutive sleep cycles across the night (Colrain, 2005). On the other hand, KC are observed in response to pathological events such as apnoea or limb movements (Karadeniz, Ondze, Besset, & Billiard, 2000). Globally, though their implications at the functional and behavioural level remain unknown, current knowledge tends to suggest a role in the synchronisation of spindles and delta waves (Adamantidis et al., 2019).

Slow waves



Description & detection

Slow waves are oscillations within the 0.5 - 4 Hz frequencies, with a minimum amplitude of 75 μ V, which are most prominent over frontal derivations (Iber et al., 2007). The term slow wave englobes two types of oscillations that are often differentiated in the literature, based on their frequency: slow oscillation (below 1 Hz), and delta waves (1 – 4Hz) (Adamantidis et al., 2019). In mice, it was shown that the lack of a certain T-type calcium channel known to be involved in brain rhythms during sleep ($\alpha 1_G$ -subunit of T-type calcium channels) leads to reduced power in the delta band, while slow oscillations remained intact (Lee, Kim, & Shin, 2004), suggesting different regulation mechanisms govern those two types of waves. Recently, the transition frequency (inverse of the time between the hyperpolarised and depolarised states) has been proposed as a novel way of distinguishing between the two types of slow waves (**figure 12**) (Bouchard et al., 2021). Based on this parameters, the authors differentiate between what they called “slow switchers” and “fast switchers”.

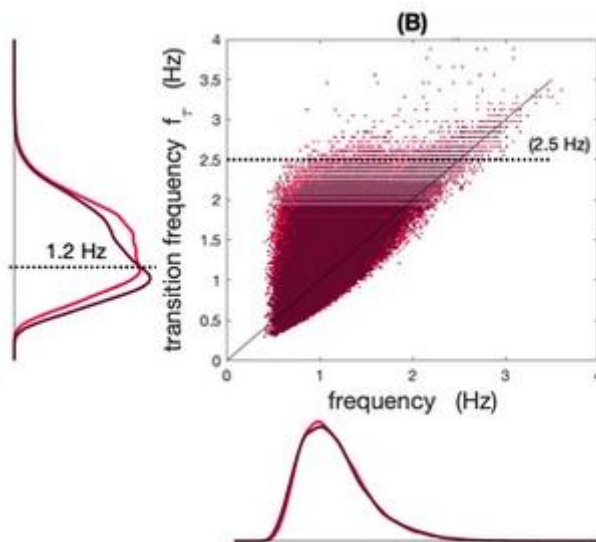


Figure 12. Frequency and transition frequency of slow waves (inverse of the time between the negative (hyperpolarisation) phase and the positive (depolarisation) phase). The scatter plot represents the exhaustive inventory of the frequency and transition frequency of all slow waves in young (light red)

and old individuals (dark red). Left side and bottom panels show the fitted gaussians on the data: while the probability distribution of the transition frequency can be related as a sum of two gaussians, the overall frequency of slow waves is modelled by only one. Reproduced with permission from (Bouchard et al., 2021)

Mechanisms

Slow waves are the resultant of thalamic and cortical neurons resting membrane potentials' variations. Delta waves have been reported to rely on thalamic neurons (Lee et al., 2004), whereas slow oscillations seem to be solely generated within the cortex, as they endure after lesion of the thalamic projections to the recorded cortical neurons (Steriade, Nunez, & Amzica, 1993; Steriade, Nuñez, & Amzica, 1993). Importantly, slow waves are travelling waves, meaning they originate in one region of the scalp and propagates, from anterior to posterior regions most frequently (Massimini, Huber, Ferrarelli, Hill, & Tononi, 2004).

Slow waves are described as having two main components: a so called “up” (active) and “down” (inactive) state, which reflect the variations in resting membrane potentials from intracellular recordings, or “on” (active) and “off” (inactive) periods which refer to the neural firing patterns from extracellular recordings, related to these up and down states (Adamantidis et al., 2019).

Influencing factors

The study of the impact of acute alcohol intake on SW yields mixed results, which are most likely due to the differences in methodology, however, SWS in alcoholic individuals were consistently reported as reduced, and so was power in the 0.75-4Hz range (Colrain et al., 2014). SW density is increased following sleep deprivation (Cajochen, Foy, & Dijk, 1999; Dijk, Beersma, Daan, Bloem, & van den Hoofdakker, 1987; Dijk et al., 1993), and conversely reduced after a nap (Werth, Dijk, Achermann, & Borbély, 1996) (cf chapter I).

Impact of ageing

Slow wave density and amplitude both decrease with age (Carrier et al., 2011). Recently, a study investigating the impact of SW amplitude detection criteria on the age-related changes in SW density showed that adapting (i.e. lowering) SW detection thresholds does not abolish the age-related decrease in SW density, while allowing to detect smaller amplitude SW produced by older individuals (T Rosinvil et al., 2021). Regarding the two types

of slow waves, ageing is associated with a reduced probability of producing fast switchers (**figure 13**) (Bouchard et al., 2021).

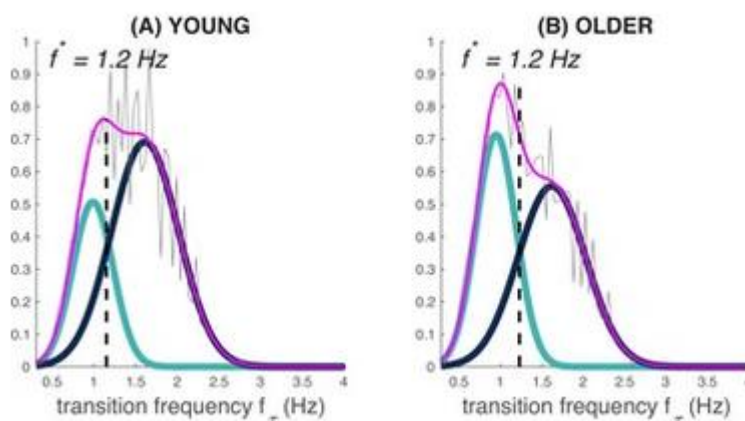


Figure 13. Normalised histograms of the transition frequency of slow waves (pink) for younger (panel A) and older (panel B) individuals, which can be decomposed into 2 gaussians, corresponding to slow (cyan) and fast (dark blue) switchers. Reproduced with permission from (Bouchard et al., 2021)

Functional correlates

The clear homeostatic regulation of slow waves elevated SWA to the rank of a marker of process S (Borbély, 2001; Vyazovskiy et al., 2009). Slow waves are also thought to play a role in information processing and neuronal interaction, as well as brain plasticity (Maquet, 2001, 2010; Miyamoto, Hirai, & Murayama, 2017). Cerebral activity during SW has been reported in various brain regions previously associated to information processing and memory consolidation (Dang-Vu et al., 2008). According to the SHY, wakefulness is associated with a net increase in synaptic strength, while sleep allows it to decrease, and SW are thought to play a role in that synaptic downscaling (Tononi & Cirelli, 2003, 2014). Interestingly, SW are hypothesised to play a role in brain maturation as well, as their topographical distribution seems to change from posterior regions in early childhood to more frontal regions in adolescence, mirroring the course of grey matter maturation (Ringli & Huber, 2011).

Slow waves and K-complexes, is it the same thing?

The intrinsic similarities between SW and KC makes it difficult to disentangle their potential different functional roles and mechanisms. Some authors postulate that the difference between SW and KC reside in the isolated and random nature of the latter (Halász, 2016).

Also, KC are often seen as forerunners of delta waves (Amzica & Steriade, 2002; Péter Halász, 2005). Halász concludes that we currently lack normative data that would allow to differentiate KC from other SW. While both these graphoelements seem to share common neurophysiological features and similar homeostatic regulation, KC seem to be linked with sensory stimulation and show high occurrence on ascending slopes of sleep cycles (Halász, 2016).

REM-alpha bursts



Description & detection

Bursts of activity in the alpha range (8-12Hz), most prominent over occipital derivations, are seen during drowsiness in wakefulness and stage NREM1 around sleep onset, but also during REM sleep, and are considered, if accompanied by an increase in muscular tone, as a sign of arousal (Cantero, Atienza, & Salas, 2002; Iber et al., 2007). Transient (<3s) alpha bursts can, however, appear during REM sleep without muscle tone increase (REM-alpha bursts), they do not constitute a sign of sleep lightening or awakening, and are rather considered an intrinsic property of the REM state (Cantero & Atienza, 2000).

Mechanisms

While waking-alpha rhythm was reported to be generated in the occipital cortex relatively independently from thalamic modulation, REM-alpha is hypothesised to be modulated by the thalamus and mediated by the reticular activating system in the brainstem (Cantero et al., 2002).

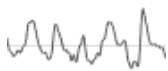
Influencing factors

Interestingly, alpha bursts seem to occur equally during phasic and tonic REM sleep, and appear to be relatively equally distributed across consecutive REM periods (Cantero, Atienza, & Salas, 2000; Cantero & Atienza, 2000).

Functional correlates

REM-alpha bursts remain an understudied feature of the human sleep, and literature dealing with this subject remains scarce. REM-alpha bursts have been postulated to represent transient temporal windows for external sensory information processing, which might account for the incorporation of external stimuli into dreams content (Cantero et al., 2002).

Sawtooth waves



Description & detection

Sawtooth waves (STW) are described as trains of 2-6Hz waves with sharp contours in REM sleep. They are most prominent over central derivations and often precede bursts of rapid eye movements (Iber et al., 2007), and are highly prevalent at the transitions between tonic and phasic REM phases (Frauscher et al., 2020).

Mechanisms

As for REM-alpha bursts, STW are a poorly understood sleep graphoelement. The origin of those waves remain to be unveiled, though they have been speculated to be related to ponto-geniculo-occipital (PGO) activity (S. Sato et al., 1997), which are spikes of activity originating from the pontine reticulum, which activate in turn cholinergic neurons in the pedunculopontine tegmentum and laterodorsal tegmentum, which in turn activate the lateral geniculate and occipital cortex (Adamantidis et al., 2019). PGO waves in REM sleep accompany eye movements (ibidem). A paper investigating the effect of a dopamine D2 antagonist (haloperidol) combined with sleep deprivation reported an increase in STW time and density with haloperidol (vs. placebo), suggesting a modulatory role of the dopaminergic system for STW generation (Pinto, Peres, Russo, Remesar-Lopez, & Tufik, 2002).

Influencing factors

A study reported no significant difference in STW density between narcoleptic patients and controls, while eye movements density was increased in the former (Geisler, Meier-Ewert, & Matsubayshi, 1987). Also, STW density is suggested to be higher from the

second sleep cycle of the night (compared to the first one) (Pearl et al., 2002). Interestingly, a study reported that STW density is bilaterally affected following unihemispheric lateral stroke (Bassetti & Aldrich, 2001).

Functional correlates

The appearance of STW on the EEG were reported to generally precede REMs (S. Sato et al., 1997). Their functional correlates remain unknown, though they have been postulated to play a role in cognitive processes during REM sleep (Frauscher et al., 2020).

Arousals



Description & detection

The definition of an arousal by the American Sleep Disorders Association (ASDA) (Bonnet et al., 1992) describes it as “an abrupt shift in EEG frequency, which may include theta, alpha and/or frequencies greater than 16Hz but not spindles”. A few further conditions are put forward: arousals can occur solely in sleep, and for that, a minimum of 10s of stable sleep (any sleep stage) is required for an arousal to be scored. It has to be highlighted that arousal scoring is independent of sleep stage scoring, which means that it can be found on an epoch that is classified as wakefulness, so long as it has at least 10s of stable sleep preceding it; and in that same vein, a minimum of 10s of stable sleep has to separate two distinct arousals, and a sleep stage change is not sufficient in itself to score an arousal unless the EEG criteria are met. Also, arousals in NREM sleep may occur with or without any concomitant increase in the EMG signal amplitude, whereas arousals in REM sleep necessitate said increase. It has to be further specified that such an increase in EMG tone is not sufficient to score an arousal, and EEG changes in the aforementioned EEG bands are a prerequisite to score an arousal.

The 3s duration criteria, though arbitrary and not physiological, is rooted in the methodological issue that concerns arousal scoring. Indeed, as arousals scoring is in part subjective as it requires an individual interpretation of what an increase in the theta, alpha or >16Hz frequencies is, the shorter the event, the lower the agreement between scorers. A study investigated the impact of arousal definition on inter-scorer reliability (Loredo, Clausen,

Ancoli-Israel, & Dimsdale, 1999), using five definitions of arousals: the ASDA definition, that is a shift in EEG frequencies involving alpha, theta or frequencies greater than 16Hz that lasts a minimum of 3s (with a mandatory increase in EMG tone in REM) – ASDA arousal; an EEG shift including alpha or theta longer lasting between 1.5 and 3s (cortical arousal 1.5); EEG shifts of duration between 3 and 15s (cortical arousal 3); EEG shift lasting between 1.5 and 15s accompanied by a submental EMG tone increase (chin EMG arousal); and EEG shift between 1.5s and 15s, with a concomitant increase in submental EMG tone as well as the presence of leg EMG activity. The last four were also categorised into events related to a respiratory event (apnoea or hypopnoea). The authors found that interscorer variability was highly variable depending on the used definition (Intraclass correlation (ICC) ranging from 0.19 to 0.92). The highest ICC was observed for arousals associated with EMG activity or leg movements, particularly if they were related to a respiratory event (ICC 0.76 to 0.92). Similar reliability was observed for ASDA arousals (ICC 0.84), and proved lowest when considering events of <3s duration (ICC 0.19 to 0.37). This all highlights the importance of the used definition, as well as its degree of interpretability, and the direct impact it has on their detection and comparability across studies.

The literature concerning arousals is very complex and heterogeneous, often based on distinct definition of arousals, but also on different arousal types. Indeed, apart from the distinction between spontaneous and elicited arousals, AASM arousals are referred to as cortical arousals, while also subcortical arousals have been described, corresponding to autonomic activation without typical EEG features of arousals (Liborio Parrino, Ferri, Bruni, & Terzano, 2012), often scored relying on autonomic activation based on heart rate changes (Togo, Cherniack, & Natelson, 2006). For the sake of clarity and in the name of simplicity, we will be focusing only on cortical (EEG) arousals.

Mechanisms

The mechanisms underlying arousals are not completely understood, and very little literature has addressed this question. One EEG study with concomitant functional magnetic resonance imaging (fMRI) reported the activation of several wake-promoting subcortical regions simultaneously with EEG arousals during NREM sleep, among which the midbrain, thalamus, basal ganglia and cerebellum, while cortical regions, including the frontal, temporal and occipital gyri were deactivated (Zou et al., 2019). However, the restricted size of the dataset (83 arousals from 19 subjects, ranging from 1 to 12 across participants) as well as

difficult data collection conditions make it hard to assess the scope and generalisability of those results, as well as to further categorise potential differences between them. Another study in zebrafish pointed towards neuronal noise in wake promoting neurons as a source of spontaneous arousals, that is the fluctuations in neuronal membrane potential, an intrinsic phenomenon, and showed that a reduction in the ambient temperature increases the rate and duration of arousals by increasing neuronal noise (Dvir et al., 2018).

Influencing factors

On the one hand, arousals are observed in high amounts in sleep related pathologies such as sleep disordered breathing (SDB) or periodic limb movements' syndrome (PLMs). On the other hand, arousals are also seen in healthy individuals' sleep, and seem to be part of sleep physiology. Due to the role they play in sleep pathologies, where they seem to constitute an alerting reaction that activates the system, in the case of sleep apnoea for instance, in order to restore breathing, they have been viewed as a vigilance mechanism with a monitoring significance (Halasz, Terzano, Parrino, & Bodizs, 2004). Nocturnal exposure to transportation noise was shown to increase the cumulative duration of autonomic arousals, without impacting sleep macrostructure (Thiesse et al., 2020).

A study in obese individuals devoid of sleep apnoea investigated arousals with and without a concomitant increase in submental EMG tone and concluded that spontaneous arousals with EMG increase were related to a higher sympathetic activation (Mendez et al., 2008), though it has to be noted that arousals in close temporal relation to a sleep stage change (<120s) were excluded.

Impact of ageing

Arousals have been reported to increase with age (Bonnet & Arand, 2007; Boselli, Parrino, Smerieri, & Terzano, 1998). When looking at the distributions of arousals across sleep stages, higher arousal prevalence was reported over the total sleep period for older individuals ($\geq 60y$) compared to all younger age groups; in overall NREM, particularly in N1 and N2, in older individuals, while no difference was found between age groups for REM sleep (Boselli et al., 1998). When separately looking at N4, teenagers exhibited higher arousal prevalence than all the older age groups; a difference that was no longer significant when combining stages N3 and N4 together (ibidem).

Functional correlates

Arousals have mostly been studied in the context of sleep pathologies (sleep disordered breathing (SDB), period limb movements' syndrome) or as elicited arousals, but rarely in the form of spontaneous arousals. In SDB, mixed results have been obtained, as their increased occurrence during the night have sometimes been related to impaired cognition, particularly attentional functions, and more rarely executive and mnesic functioning, while other studies imputed those cognitive alterations to brain hypoxia (Aloia, Arnedt, Davis, Riggs, & Byrd, 2004). Studies on experimentally induced arousals, mostly involving auditory stimulations, also lead to mixed outcome, as some reported an impact on subsequent daytime alertness as well as sleep propensity (Roehrs, Merlotti, Petrucelli, Stepanski, & Roth, 1994) as well as sleep propensity (Martin, Engleman, Deary, & Douglas, 1996; Philip, Stoohs, & Guilleminault, 1994) where others failed to find an impact on cognition (Kingshott, Cosway, Deary, & Douglas, 2000; Martin et al., 1996; Philip et al., 1994). A link between higher arousal index and increased morning cortisol levels, heart rate, blood pressure and cholesterol levels has also been put forward (Ekstedt, Åkerstedt, & Söderström, 2004).

Cyclic Alternating Pattern (CAP)

Description & detection

The Cyclic Alternating Pattern (CAP) is a periodic activity, found in states of reduced vigilance such as sleep or coma (Parrino & Terzano, 2017), that is characterised by the intermittent alternation between distinct EEG patterns (phase A and phase B, **Figure 14**), reflecting different levels of arousal response to stimulation (Terzano et al., 1985). It is reported as an event occurring during normal sleep. The authors found that when presented with an auditory stimulus during CAP, the pattern observed in phase B changes into the one observed in phase A, while presenting a stimulus during phase A never caused the EEG pattern of phase B. They concluded that phase A represents a higher arousal level, and phase B a lower one (ibidem).

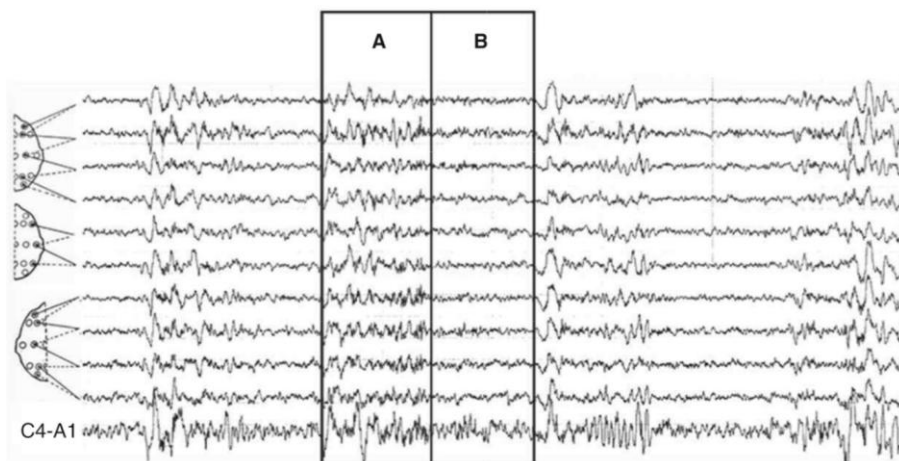


Figure 14. CAP sequence in the EEG – Phase A (composed here mostly of K-complexes) and B (interval between successive phases A). Reproduced with permission from (L Parrino & Terzano, 2017).

The alternation of a phase A followed by a phase B constitutes a CAP cycle, and at least two consecutive CAP cycles form a CAP sequence. Each CAP phase (A or B) range from 2 to 60s in length. A period longer than 60s which shows the absence of CAP is considered as non-CAP, and reflects an period of sustained physiological stability (Parrino & Terzano, 2017).

Importantly, according to this theory, arousals are not only considered as increases in EEG frequencies (as ASDA/AASM arousals), but can also relate to frequency decreases, and thus be associated to a deepening of sleep (Bonnet et al., 2007). Indeed, 3 types of phase A have been distinguished: type A1, defined as a predominance of slower frequencies (delta bursts, K-complex sequence or vertex waves); type A3 is characterised by a predominance of higher frequencies; while type A2 is a combination of slower and faster rhythms (Parrino & Terzano, 2017). Classically understood arousals relate thus to type A3 of the CAP pattern.

Interestingly, it was observed that CAP sequences are always present in the NREM part preceding a REM period, and conversely, are always absent in the NREM periods directly following REM periods (Terzano et al., 1985).

Mechanisms

Similarly to arousals, the mechanisms behind CAP sequences are not well studied. As CAP sequences are constituted of distinct phases, it is likely that each phase has a different

substrate. In general, cortical-brainstem interplay has been reported as the originating mechanism of CAPs (Terzano & Parrino, 2000).

Influencing factors

CAPs are generally thought to be a feature of NREM, and to be absent in healthy REM sleep, while their appearance in that stage can be characteristic of sleep pathologies, such as periodic REM-related sleep apnoea events (Parrino et al., 2012). CAP rates are also impacted by sleep deprivation, as the recovery night after 40h of continuous wakefulness was reported to contain significantly less CAP events compared to baseline (De Gennaro et al., 2002). Not surprisingly, the sleep of patients with depression is described as containing higher CAP rates compared to healthy controls (Farina et al., 2003).

Impact of ageing

Studies reported a U-shaped distribution of CAP time (relative to total NREM time) across the life span, with higher values in teenagers (~43%), progressively decreasing in early adulthood (~32%) then slow increases of those rates in middle age (~37%) and older individuals (~55%) (Migueis et al., 2021; Parrino, Boselli, Spaggiari, Smerieri, & Terzano, 1998). Different aspects of CAP behave differently between age groups, with higher rates of A1 subtypes in teenagers, and higher rates of A2 and A3 subtypes, along with a longest duration of CAP cycles in older individuals (ibidem).

Functional correlates

Globally, CAPs are considered as an EEG marker of sleep instability (Parrino et al., 2012; Terzano & Parrino, 2000). As such, they have been linked to cognitive processing during sleep, as A1 subtype was shown to have increased rates in post-learning sleep, which correlated with the performance improvement (Ferri et al., 2008). CAP rates of phase A1 were also shown to correlate with divergent thinking (Drago et al., 2011), verbal fluency, and working memory (Novelli, Ferri, & Bruni, 2013), while those of phases A2 and A3 have been associated to worse planning performance. It seems therefore that the correlate of CAPs depend on the investigated subtype and the main type of oscillation they contain.

Conclusion

In summary, graphoelements interwoven in the sleep EEG are complex patterns which mechanisms and functional role are still being investigated. As we have seen, some

graphoelements contribute to transferring information during sleep, some are thought to play a sleep protecting factor or contribute to synaptic downscaling, while the function of others remain still unclear. Importantly, while many studies focused on unravelling the primary functions of those waveforms, it is only relatively recently that research has started to lean on the coalescence of those different elements, which might be as important as those features themselves. For instance, spindles are often times coupled to slow waves up state (Klinzing et al., 2016; Mölle, Bergmann, Marshall, & Born, 2011). Recent research is showing that the coupling of slow oscillations and spindles changes across the lifespan, with its strength increasing from childhood to adolescence, mirroring the increment observed in memory formation (Hahn, Heib, Schabus, Hoedlmoser, & Helfrich, 2020), while its temporal organisation, compromised in ageing, seems to at least partly support the age-related decrease in memory retention (Helfrich, Mander, Jagust, Knight, & Walker, 2018).

In the context of this dissertation, we will focus mainly on the cognitive and brain correlates of arousals, in the first results section. In the second results chapter, we will investigate the cerebral microstructure associated to precise slow wave characteristics. In the third chapter, we will explore the relationships between slow waves-spindle coupling and cortical amyloid burden and cognitive decline.

Chapter III: Cerebral and cognitive ageing

In this chapter, we will give a brief overview of the changes that characterise normal ageing, on the cognitive and cerebral levels. We will then focus on pathological ageing, describing dementia, with a particular emphasis on Alzheimer's disease (AD), which is the most prevalent cause of dementia. We will describe the cerebral changes that characterise this pathology, i.e. extracellular aggregation of amyloid-beta ($A\beta$) protein, neurofibrillary tangles of tau and neurodegeneration. We will finish by reviewing the current knowledge about the relationship between the hallmarks of AD and sleep and wakefulness regulation.

Normal ageing

Cognition

Normal ageing, defined as the changes occurring in individuals devoid of diseases affecting the nervous system (Dumas, 2015), is characterised by a series of changes at the cognitive level, and affects several of its aspects (Harada, Natelson Love, & Triebel, 2013; Mielke et al., 1998; Toepper, 2017). Processing speed has been reported as decreased from as early as the third decade of life (Harada et al., 2013; Mielke et al., 1998). Declining memory is among the most frequent cognitive complaints among older individuals (Craik, 2008; Dumas, 2015). As memory is not a unitary system, therefore not all types of memory and not all its processes are uniformly altered. In declarative (or explicit) memory, episodic (autobiographical) memory was reported to decrease throughout the lifespan, while semantic (general knowledge) memory is reportedly relatively preserved (Craik, 2008) or impaired quite later in life, and procedural memory is spared by the ageing process (Craik, 2008; Harada et al., 2013). Regarding the different processes, the acquisition of novel information, as well as its retrieval both decline with age, while memory retention in itself seems stable (ibidem). This means that tasks involving free recall of information are usually performed more poorly, while those based on recognition show relatively preserved performance (Mielke et al., 1998). Attentional processes seem to undergo a slight decline, where more complex aspects of attention are particularly impacted, for instance selective and divided attention generally decrease (Harada et al., 2013). Concerning executive functioning, flexibility has been reported to decrease from ~70y, working memory also declines with age (Craik, 2008), while inhibition is not systematically described as impacted (Collette & Salmon, 2014). It has been reported that older individuals show increased activation of prefrontal areas on executive tasks compared to young, hypothetically as a compensation mechanism (Collette & Salmon, 2014;

Dumas, 2015; Turner & Spreng, 2012). Importantly, the inter-individual variability in cognitive abilities in elderly populations is substantial, as some individuals seem to show preserved functioning relatively late, while other individuals seem to exhibit cognitive decline early on (**figure 15**) (Juan & Adlard, 2019; Nyberg, Lövdén, Riklund, Lindenberg, & Bäckman, 2012).

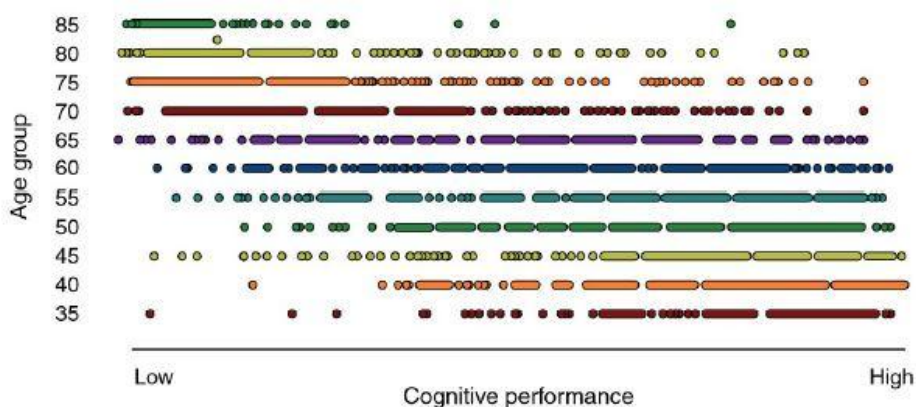


Figure 15. Graphical illustration of the variability in memory performance across age groups. Although a general trend for lower performance in older individuals can be seen, some individuals retain high level performances. Reproduced with permission from (Nyberg et al., 2012)

Cerebral ageing

Ageing is also characterised by changes in structural brain integrity. The overall volume of the brain reportedly decreases by 0.2-0.5% per year (Fjell & Walhovd, 2010). Studies have reported a global reduction in cerebral blood flow and metabolism in older individuals compared to young, which was particularly visible over the frontal regions (Mielke et al., 1998). Grey matter volume starts decreasing as early as the third decade of life, its most prominent changes being observable in the prefrontal cortex, with moderate changes in the temporal lobe, mostly in the hippocampus (Toepper, 2017). The entorhinal cortex, which relays information between the hippocampus and associative regions of the brain, is impacted early on in the process of Alzheimer's disease (AD), but not in normal ageing (Harada et al., 2013). This grey matter volume decrease has been reported to be best explained by a reduction in neuronal size and density of connections, more than in their death (Dickstein et al., 2007; Harada et al., 2013). Normal ageing is associated with a reduced complexity of the dendritic arborisation (Dickstein et al., 2007). The decrease in white matter volume is reportedly far greater than that of grey matter (Harada et al., 2013), caused by demyelination (Toepper,

2017). Unlike grey matter that exhibits a rather linear decrease from early adulthood to old age, white matter volume seems to follow an inverted U-shape, first increasing from childhood to adulthood, before progressively decreasing (Toepper, 2017). Anterior regions seem to exhibit higher white matter degeneration which has been related to impaired executive functioning (Harada et al., 2013).

Dementia

Dementia is a syndrome characterised by cognitive decline, usually accompanied by behavioural and mood impairments, with impact on the individual's day-to-day functioning (Gale, Acar, & Daffner, 2018). Though some causes of dementia may be non-neurodegenerative (and thus, at least theoretically, reversible), dementia is most often caused by neurodegeneration and is therefore progressive (Ljubenkov & Geschwind, 2016).

At the behavioural level, dementia syndrome can be quite heterogeneous, and can be characterised by amnesia, apraxia, aphasia, or a dysexecutive syndrome (Ljubenkov & Geschwind, 2016; Toepper, 2017). At the neurological level, depending on the cause of dementia, symptoms may appear quite late and be relatively unspecific, while for some types (subcortical), they can play a primary role in the diagnosis. At the social level, they are characterised by the progressive inability to get involved in daily tasks and family life, thus by a progressive loss of autonomy (Toepper, 2017).

Alzheimer's disease

The most prevalent cause of dementia (from 50-80%), especially in the elderly, is AD (Walker, McAleese, Erskine, & Attems, 2019). Two forms of AD can be distinguished: a sporadic (~99% of cases), and a familial form (Bekris, Yu, Bird, & Tsuang, 2010; Lista et al., 2015). The sporadic form is not hereditary, though the presence of a certain gene mutation (e.g. in apolipoprotein E protein) constitutes an increased risk of developing AD (Parvathy & Buxbaum, 2002).

The development of AD can be divided in three clinical stages (Jack et al., 2013). Firstly, a presymptomatic phase, also called preclinical AD, where brain pathological changes start taking place, but the individual is still cognitively normal. The second phase, or prodromal phase, also called mild cognitive impairment (MCI) is characterised by the emergence of the first cognitive symptoms (classically episodic memory difficulties). The final phase of AD is dementia, characterised by such severe and broad cognitive symptoms that they impact daily

functioning and render the individual unable to live independently. Among AD dementia, several types can be distinguished based on their symptomatology, including typical and atypical AD, with non-amnesic variants (such as logopenic variant primary progressive aphasia, or posterior cortical atrophy) which can cause diagnostic difficulties (Mendez et al., 2019). As those are much more prevalent among early-onset (familial) patient, and thus represent only a small percentage of AD cases, we will focus on typical AD.

Cognition

Among the first cognitive symptoms of typical AD are episodic memory deficits (Craik, 2008), with poor retrieval but also poor recognition performances. Those impairments can be explained by a defective encoding, which jeopardises further memory processing (Toepper, 2017). Access to semantic memory is also compromised (Verma & Howard, 2012), leading to struggle in finding words, reflected by poor performance on naming tasks.

Cerebral changes in AD

As life expectancy is continuously increasing, so is the incidence of Alzheimer's disease (AD). It has reached the seventh position of the most important causes of death worldwide, according to the World Health Organisation (WHO)¹. As the search for efficient treatment is failing to find therapies which would satisfyingly revert or delay disease progression, the most promising approach resides in early detection and prevention. Some estimate that delaying AD onset by only 5 years would decrease its costs by about 40% (Zissimopoulos, Crimmins, & Saint Clair, 2015). Therefore, an increasing part of the literature has been focusing on early biomarkers which would help understand the pathophysiology of the disease, and hopefully, prevent or at least delay the onset of the symptoms.

It has to be pointed out that the clinical diagnosis of AD is made based on the clinical picture, which can be confirmed by autopsy. Recently, the USA National Institute on Aging and Alzheimer's Association (NIA-AA) proposed an update in the definition of AD for research, which is based on the underlying pathological processes that occur in the brain, shifting therefore from a clinical to a biological approach (Jack et al., 2018). So far, three main biological markers of AD have been put forward: extracellular beta-amyloid plaques (A β), intracellular neurofibrillary tangles (NFT) made up of hyperphosphorylated tau protein, as well as neurodegeneration (Jack et al., 2018). These biomarkers constitute relevant factors in

¹ <https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death>

predicting the evolution across the AD continuum, for the conversion of prodromal AD to fully declared AD but also from preclinical to MCI (Jack et al., 2013; Roe et al., 2013). Crucially, these pathophysiological processes are now known to start decades before the symptom onset. Though their presence does not by itself predict a future conversion to AD, they represent an increased risk for future development of the disease, together with apolipoprotein ϵ (APOE) polymorphism.

Amyloid- β

A β designates peptides of amino acids, with species varying in length at least from 36 to 43 (Masters & Selkoe, 2012). Though several species of various length exist, the most well-known are the most abundantly produced species, A β_{40} (~80-90%), and the far more scarce A β_{42} (5-10%), as they play a major role in the formation of amyloid plaques because of their hydrophobic and fibrillogenic properties (Kim et al., 2007; Lambert et al., 1998; Murphy & LeVine III, 2010). The physiological function of A β remains unclear but is thought to play a role in normal synaptic function (Masters & Selkoe, 2012). Soluble oligomeric assemblies of A β have been suggested as toxic (Xekardaki et al., 2014).

A β is derived by the cleavage of the amyloid- β precursor protein (APP) (Walker et al., 2019), and mutations in APP have been related to an increased risk of developing AD (Parvathy & Buxbaum, 2002). It is thought that the beginning of AD pathophysiology is related to abnormal processing of APP, which then leads to increased production/reduced clearance of A β in the cortex (Hardy & Selkoe, 2002).

A β plaque deposition can be monitored via cerebrospinal fluid A β_{42} quantification, as decreased A β_{42} concentration correlate with *post-mortem* A β pathophysiology (Clark et al., 2003; Strozzyk, Blennow, White, & Launer, 2003). Low CSF A β_{42} concentrations are thought to echo, and even precede amyloid plaque formation (Palmqvist, Mattsson, & Hansson, 2016; Sprecher et al., 2017). Over recent years, labelling brain amyloid deposits *in vivo* became possible with PET using various compounds binding to β -sheets of aggregated amyloid proteins (e.g., 11C-Pittsburg compound B (PiB), 18F-flutemetamol). These compounds were shown to bind to neuritic plaques, less so to diffuse plaques and marginally to NFT (Ikonomovic et al., 2008; Lockhart et al., 2007). The semi-quantitative estimation of their brain binding, usually associated with a binary decision (Ab+ vs Ab-), was shown to identify brain amyloid deposits in patients with clinically overt AD disease (Klunk et al., 2004) with excellent (>90%) sensitivity and specificity (Devanand et al., 2010). Positive PiB amyloid

imaging and abnormally low CSF $A\beta_{42}$ have been shown to correlate almost perfectly in patients tested by both (Fagan et al., 2006; Grimmer et al., 2009; Jagust et al., 2009)). Recently, plasma markers of $A\beta$, obtained by blood test, have been developed as they offer a non-invasive and cost-effective way of assessing $A\beta$ levels, but unfortunately might lack specificity (Verberk et al., 2018).

The pathophysiological process of $A\beta$ deposition has been proposed to follow a stereotyped inwards pattern (**figure 16**). Its progression has been subdivided in five stages (Thal, Rüb, Orantes, & Braak, 2002). In the first stage, $A\beta$ deposits can be found in the neocortex. Next stages involve allocortical regions (entorhinal cortex, hippocampus); then progressively the basal forebrain is reached. $A\beta$ deposition then encompasses various brainstem nuclei, and ultimately, the cerebellum.

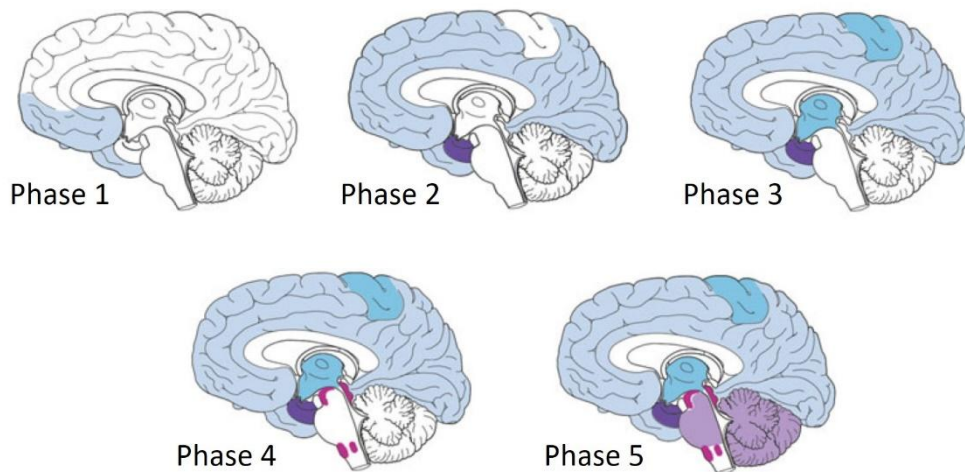


Figure 16. Sequential phases of $A\beta$ deposition, adapted with permission from (Heiko Braak & Del Tredici, 2015)

Tau

Tau designates a microtubule-associated protein, involved in maintaining axonal cytoskeleton stability (Mietelska-Porowska, Wasik, Goras, Filipek, & Niewiadomska, 2014). However, due to abnormal hyperphosphorylation, oligomerisation, tau then changes into insoluble filamentous state (Nelson et al., 2012); after which it binds into toxic oligomers and eventually takes the form of intracellular neurofibrillary tangles (NFTs) (Gómez-Ramos, Díaz-Hernández, Cuadros, Hernández, & Avila, 2006).

Increased CSF tau has been shown to correlate with *post-mortem* brain NFTs (Tapiola et al., 1997). Unfortunately, as for A β , though CSF values remain an important indicator of tau pathological processes, it does not allow to assess the spatial distribution, which is why PET radiotracers targeting tau are increasingly investigated. Though first generation tau radioligands have reportedly high *in vitro* affinity and selectivity (Lemoine et al., 2017), some off-binding to monoamine oxidase B (MAO-B) was found *in vivo*, particularly over the basal ganglia (Chiotis et al., 2018). As for A β , recent development of plasma markers of levels of tau, phosphorylated tau 181 (ptau181), ptau217 and ptau231 may constitute clinically promising new tools (Blennow, 2021; X. Ding et al., 2021).

While longitudinal and larger cohort studies are further needed to confirm their validity, second-generation radiotracers seem to show little off-target binding, and the third-generation ones appear even more promising (Bischof et al., 2021; Mittal et al., 2021).

Similarly to A β , the pathophysiological process of tau follows a characteristic progression pattern (**figure 17**) (Braak & Braak, 1991). This progression has been divided into stages (preliminary stages a-c and proper stages I to VI). The first intraneuronal lesions can be observed in subcortical nuclei, mostly in the LC (Braak, Thal, Ghebremedhin, & Del Tredici, 2011), as early as pre-puberty/young adulthood (stage a). Over 2 332 non-selected autopsied bodies ranging from 1 to 100 years, the authors found only 10 (0,4%) were completely devoid of abnormally phosphorylated tau protein lesions, all of them were younger than 23 yo. Stages b and c are characterised by the appearance of pretangle and tangle material in coeruleus neurons' soma and dendrites, as well as in other brainstem nuclei with diffuse cortical projections (Braak & Del Tredici, 2015). With stage progression, tau pathology slowly occurs in transentorhinal regions (from stage 1a), then reaches neocortical association areas before finally being found in primary and secondary areas (Braak & Del Tredici, 2015; Braak et al., 2011).

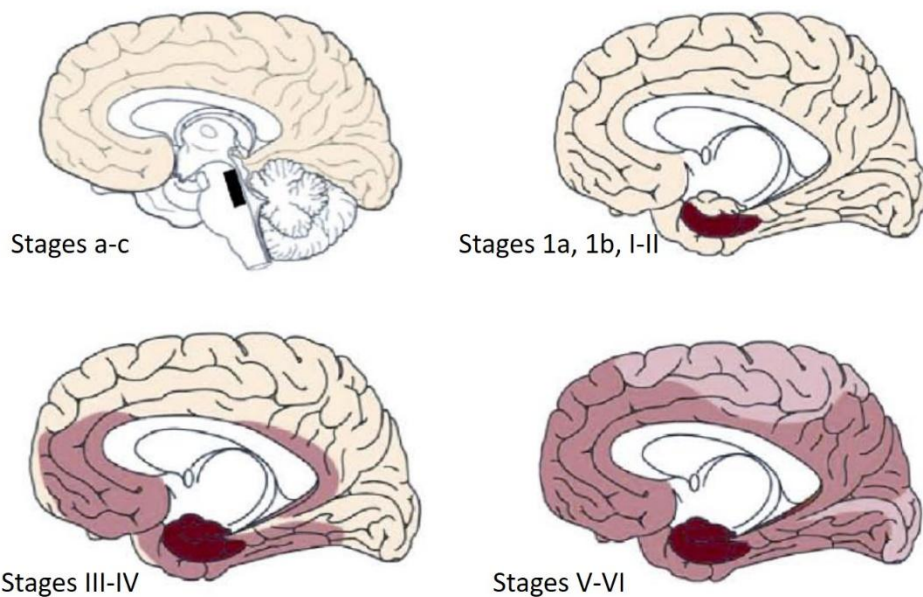


Figure 17. Stages of tau deposition, adapted with permission from (Heiko Braak et al., 2011)

Neurodegeneration

Neurodegeneration is the process of neuronal and synaptic loss. Of the three hallmarks of AD, it is the one most strongly correlating with dementia symptoms (Jack et al., 2010). The early neurodegeneration associated with AD mirrors that of early NFTs accumulation before encompassing temporal and parietal cortices, and frontal regions in later stages (Thompson et al., 2003). The first signs of atrophy mainly cover the entorhinal cortex and the hippocampus, before spreading out (Fjell, McEvoy, Holland, Dale, & Walhovd, 2014). The level of atrophy in medial temporal regions is a diagnostic marker for the MCI stage of AD (Frisoni, Fox, Jack, Scheltens, & Thompson, 2010).

The amyloid cascade hypothesis

A model of a hypothetical pathophysiological cascade has been proposed, where key biomarkers of AD are thought to occur in a temporally ordered manner (**Figure 18**) (Jack et al., 2013, 2010). This model is of crucial importance as neither A β plaques nor tau aggregations alone allow to account for AD symptoms. It has been proposed that it is the abnormal processing of A β leading to A β plaques formation in the brain that initiates a set of pathological processes that include neuronal injury and neurodegeneration (Hardy & Higgins,

1992). The earliest manifestations can be observed in abnormally low CSF A β_{42} and via amyloid PET imaging, those are followed by CSF tau and FDG PET, then structural MRI, and only thereafter do clinical symptoms become apparent. In fact, CSF A β_{42} was shown to reach abnormal levels 5-10 years or more prior to dementia diagnosis (Buchhave et al., 2012). Some results suggest CSF A β originally reached abnormally high concentrations before progressively declining to low values (Bateman et al., 2012). Data collected by Bateman and colleagues also suggests that tau reaches abnormal values before FDG PET, which in turn becomes abnormal with MRI values in about the same timeframe (ibidem). Though some debate exists as to whether A β or tauopathy are the starting point of AD neuropathological processes, the model proposes that while tau-related neurodegeneration would be the first change occurring in the brain, but, as this change is only detectable by immunostaining, it remains below detection threshold, explaining why most studies reported an earliest A β process (Jack et al., 2013). Thus, it is hypothesised that the abnormal processing of A β , leading to its accumulation in the brain (either through enhanced production or reduced clearance) (Jack et al., 2010), initiates the pathological cascade of AD, by facilitating or precipitating neuronal death and NFT formation (Hardy & Higgins, 1992), through mechanisms that haven't yet been identified.

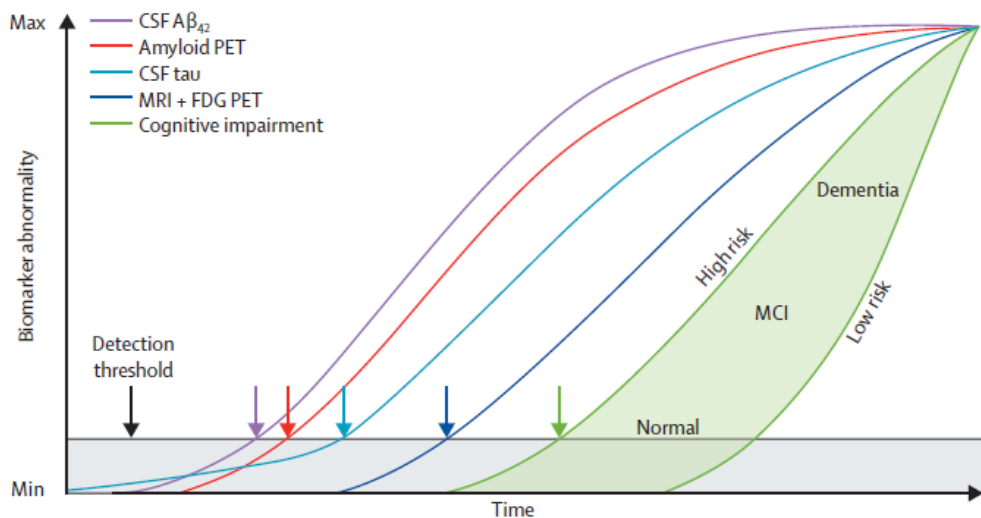


Figure 18. Model of the pathophysiological changes occurring in the brain in Alzheimer's disease. The horizontal line indicates the threshold below which the changes go undetected. Changes in tau levels are thought to precede A β deposition, but only early on below this detection threshold. A β is then thought to rise above detection threshold, as detectable first via CSF (purple line) then PET (red line). These changes in turn are thought to accelerate tauopathy, with detectable CSF levels (light blue line).

line). Later on, MRI and FDG PET markers rise above detection threshold (dark blue line). Cognitive impairment are the last observable changes. Reproduced with permission from (Jack et al., 2013)

It is important to note that A β dysfunction, leading to A β plaque formation, is a prerequisite, but is not enough in itself to produce clinical AD symptomatology. The severity of A β deregulation as observed through CSF A β ₄₂ levels or A β PET imaging only loosely correlates with the severity of cognitive decline, the latter being more linked to tau/neurodegeneration biomarkers (Bennett, Schneider, Wilson, Bienias, & Arnold, 2004).

Sleep and AD biomarkers

A β , tau and sleep in animal models

It has been reported that about 30% of AD cases could be attributable to modifiable risk factors, such as lack of physical activity, diabetes, or midlife hypertension (Norton, Matthews, Barnes, Yaffe, & Brayne, 2014). It appeared therefore of crucial importance to identify other potentially modifiable risk factors in order to reduce AD incidence or at least delay disease progression. One such factor is sleep, as a recent meta-analysis reported that approximately 15% of AD cases were imputable to sleep problems (Bubu et al., 2018). Over recent years, an increasing set of data has thus been focusing on unravelling the link between sleep and AD (Van Egroo, Narbutas, Chylinski, Villar González, Maquet, et al., 2019a). One key report that contributed massively to establishing the relevance of this link was made by Kang and colleagues, which showed that interstitial fluid (ISF) levels of A β show a diurnal variation, as, in mice, ISF A β levels are reduced by about ~25% during the light period (corresponding to their period of sleep) relative to the dark period; a fluctuation that was still observable even during constant dim light condition (Kang et al., 2009). This diurnal fluctuation of A β levels was thus established as an intrinsic property of normal cellular physiology. Since then, several other animal models have confirmed a link between sleep and AD biomarkers. It was shown that acute sleep deprivation and chronic sleep restriction lead to increased A β and tau levels in the brain and the CSF (Chen et al., 2017; Di Meco, Joshi, & Praticò, 2014; Kang et al., 2009; Roh et al., 2014; Rothman, Herdener, Frankola, Mughal, & Mattson, 2013). Increased levels of tau in the brainstem (Holth, Mahan, Robinson, Rocha, & Holtzman, 2017) and in the cortex/hippocampus (Jyoti, Plano, Riedel, & Platt, 2015; Platt et al., 2011) as well as increased A β burden in the hippocampus (Roh et al., 2012) have all been linked to higher amounts of wakefulness and decreased NREM/REM duration during the

sleeping period. Importantly, the release of A β was shown *in vivo* to be driven by neuronal activity (Cirrito et al., 2005).

A β , tau and sleep in humans

In humans, longer self-reported sleep latency, shorter sleep duration and poorer sleep quality are all associated with higher cortical and hippocampal/thalamic A β burden (Branger et al., 2016; Brown et al., 2016; Insel, Mohlenhoff, Neylan, Krystal, & Mackin, 2021; Shokri-Kojori et al., 2018; Spira et al., 2013) and abnormal CSF A β levels (Sprecher et al., 2017) in cognitively normal individuals. Increased daytime sleepiness was also related to abnormal CSF A β levels (Sprecher et al., 2017), higher cortical A β levels (Sprecher et al., 2015) and longer longitudinal A β accumulation (Carvalho et al., 2018; Spira et al., 2018), while self-reported daytime sleep was also associated with higher brain A β deposition in amyloid-negative individuals (i.e. in individuals where A β is present in low amount, below a clinical threshold) (Insel et al., 2021). In older individuals (70-85y) with subjective memory complaints but no objectively detected memory deficits, A β positive individuals had significantly higher sleep latency and wake after sleep onset as well as lower sleep efficiency, measured via actigraphy (Ettore et al., 2019). Lower actigraphy-derived measured sleep efficiency was also related to abnormal CSF A β values (Ju et al., 2013), and a higher fragmentation of the rest-activity cycle was related to higher cortical A β burden (Musiek et al., 2018). A lower SWA recorder by polysomnography has also been related to higher A β levels, whether in the CSF (Varga et al., 2016) or measured by PET scan in the medial prefrontal cortex (mpfc) (Mander et al., 2015). Experimental manipulations, such as sleep deprivation were shown to result in higher CSF A β levels (Blattner et al., 2020; Lucey et al., 2018; Ooms et al., 2014), as did specific SWA disruption (Ju et al., 2017).

Tau protein has also been related to sleep in humans, as worse subjective sleep quality has been associated with higher CSF tau levels (Sprecher et al., 2017), sometimes only in A β positive individuals (Fjell et al., 2018). Increased daytime sleepiness was also related to elevated CSF tau levels (Sprecher et al., 2017). Home sleep quality, sleep consolidation, and fragmentation of rest-activity cycle measured by actigraphy were also all related to CSF tau levels or *post-mortem* NFT density (Ju et al., 2017; Lim, Kowgier, Yu, Buchman, & Bennett, 2013; Musiek et al., 2018). In animal models, ISF tau levels were increased by about 50% during wakefulness as compared to sleep, as well as during sleep deprivation (Holth et al., 2019).

A β and tau in sleep pathologies

Similarly, studies on sleep related pathologies seem to confirm a link between sleep processes and AD pathophysiology. A study by Elias and colleagues (Elias et al., 2018) found that OSA was associated with higher brain A β burden, but that this association does not survive when they controlled for BMI or APOE ϵ 4, for which values and incidence were both higher in the OSA group compared to controls. Other studies found that higher apnoea/hypopnoea indices were related to abnormal CSF A β levels and higher longitudinal A β accumulation (Ju et al., 2016; Sharma et al., 2018), and higher serum and plasma tau levels (Bu et al., 2015; Motamedi et al., 2018). Higher A β burden have been reported in OSA patients compared to control, and was significantly higher in patients with severe OSA compared to mild/moderate OSA syndrome (Jackson et al., 2020). A study investigating the impact of OSA treatment determined that it increased SWA, which in turn correlated with lower CSF A β values after treatment (Ju, Zangrilli, Finn, Fagan, & Holtzman, 2019). Generally, OSA is considered as an increased risk factor for potential future development of AD (Bubu et al., 2020, 2018; Ylä-Herttuala et al., 2021).

Neurodegeneration and sleep

Synaptic and neuronal loss are among the best pathological correlates of dementia, and are an important predictor of conversion from MCI to AD (Bouwman et al., 2007). Two studies in mice have reported a link between extended wakefulness/chronic sleep disruption and a loss of neurons in the locus coeruleus (Zhang et al., 2014; Zhu et al., 2007). In humans, higher sleepiness has been linked to lower cortical thickness (Carvalho et al., 2017), and higher self-reported sleep quality has been related to higher white matter integrity (Takeuchi et al., 2018) and lower grey matter atrophy rate (Sexton, Storsve, Walhovd, Johansen-Berg, & Fjell, 2014). Higher rest-activity cycle fragmentation has been associated to higher medial temporal lobe atrophy (Van Someren et al., 2018). Some studies showed that sleep duration is relevant for atrophy, notably the annual rate of ventricles expansion (Lo, Loh, Zheng, Sim, & Chee, 2014) and longitudinal rate of atrophy (Spira et al., 2016). Slow wave density and amplitude have also been reported to be related to grey matter integrity in SW-related regions (Dubé et al., 2015).

The glymphatic system

Potential mechanisms explaining those findings are still being investigated. One important process, particularly for A β aggregation, is related to the so called glymphatic system, which was recently discovered (Plog & Nedergaard, 2018; Xie et al., 2013). The glymphatic system is a macroscopic waste clearance system, with a function of eliminating soluble proteins and metabolic waste in the CNS (Jessen et al., 2015), akin to the role of the lymphatic system for peripheral tissues. The CSF and ISF are in continuous exchange, which is facilitated by convective influx of CSF along the periarterial space (Iliff et al., 2012). CSF movement along the periarterial space drives convective ISF fluxes, which are in turn exited out of the brain to the cervical lymphatic system (Jessen et al., 2015). The name glymphatic is meant to recall the similarities to the lymphatic system, as well as the important role of glial cells in the convective fluid transport. Importantly, it was reported that sleep is related to an increase ~60% in the interstitial space volume, which leads to an easier glymphatic clearance (Xie et al., 2013).

The importance of the glymphatic system for neurodegenerative diseases such as AD has been shown by Iliff and colleagues. By injecting fluorescent amyloid β_{1-40} into mice striatum and showing it was rapidly cleared from the mouse's brain along the glymphatic pathway, they highlighted the role of the glymphatic's clearing abilities is surely non negligible in the accumulation of those proteins. A series of research has shown that glymphatic activity could be related to breathing, as CSF movement has been related to respiration (Klose, Strik, Kiefer, & Grodd, 2000) and arterial pulsatility. Furthermore and importantly, the glymphatic system activity is related to sleep. Indeed, CSF influx was shown to be reduced by at least 90% during wakefulness compared to anesthetised mice, which was then confirmed in naturally sleeping mice (Xie et al., 2013). This led to conclusion that sleep is of high importance for the glymphatic system which seems to be more active in that state, and highlights its crucial role for the clearance of metabolic waste. Moreover, noradrenaline, a key neuromodulator involved in arousal state, might be involved in the glymphatic activity as administration of noradrenaline antagonist in mice during wakefulness resulted in increased CSF influx comparably to that in the sleeping state, while administration of noradrenaline decreased interstitial volume fraction (Xie et al., 2013). Importantly, a recent paper showed a precise sequence between the occurrence of a slow wave on the EEG, the BOLD signal and CSF flux, meaning that the glymphatic system and slow waves would be linked through hemodynamic response (Fultz et al., 2019), though the underlying mechanisms are currently not well understood.

Interestingly, glymphatic activity is greatly reduced in aged mice compared to their young counterparts (Kress et al., 2014), which might be partly imputable to dysregulation of astroglial water transport, or to a reduced production of CSF and CSF pressure (Jessen et al., 2015), as well as reduced flexibility of arterial wall which in turn reduces arterial pulsatility (Iliff et al., 2013). This is of high importance for the understanding of neurodegenerative disease for which ageing is the highest risk factor. The reduced activity of the glymphatic system might provide insight into the understanding of neurodegenerative disease, as all prevalent neurodegenerative diseases are related to the accumulation of aggregated proteins (Ross & Poirier, 2004). Though evidence in rodents as well as humans suggest that metabolite waste clearance in the brain are an intrinsic fragility found across species, studies on the glymphatic system in humans need yet to corroborate its existence and functioning.

Conclusion

Together, all those findings indicate a role for sleep-wake regulation in the pathophysiological processes related to AD in its preclinical stages, many years before cognitive symptoms become apparent. Evidence is pointing towards a bidirectional relationship with all three hallmarks of AD, though the investigations related to A β remain more advanced. Recent advances in techniques represent an incredible opportunity to further the understanding we have of the pathology, whether through increasingly sensitive MRI at 7 Tesla and objective quantitative MRI, or the development of highly sensitive PET radioligands, especially in the case of third generation radiotracers for tau imaging. It seems that the large time-window of the progression of AD pathophysiology is a unique occasion for potential sleep-related interventions, which could possibly reverse or at least delay disease progression, though we are still relatively early in the unravelling of the mechanisms linking sleep to AD hallmarks, and the beneficial impact of improving sleep remain to be proved.

Study objectives

In the previous sections, we saw how sleep plays a key role for several physiological and behavioural functions. We also saw how alterations in sleep quality are an inherent part of the ageing process, with, at the macrostructural level, a lower depth of sleep, as observable through less time spent in NREM3 and more frequent awakenings, but also microstructural aspects being affected, among which slow waves, spindles and arousals, which seem to play an important role for cognition. An increasing amount of data is pointing towards a key role for sleep in the cognitive trajectory and pathophysiological processes occurring in the brain.

The general aim of the present dissertation was to investigate, in a healthy population, the characteristics of sleep which are associated to increased risk factors for the future development of AD, in the form of its increased biomarkers. We also intended to examine the sleep features that would be associated to poorer cognition, as well as to poorer cognitive trajectory on a 2 years longitudinal assessment. As sleep macrostructure is composed of finer, dynamical aspects of microstructural elements, we hypothesised that those would be the earliest to exhibit modifications, before an impact could be detectable over other, less refined aspects of sleep. Therefore, the main protagonists of this work are sleep microstructural waveforms.

But, before undertaking that task, we addressed some issues related to methodological aspects. In the first methodological article, we discuss the difficulty of sleep stage scoring in elderly populations. For that aim, we used the scoring of a previously validated sleep scoring algorithm (Berthomier et al., 2007; L Peter-Derex et al., 2020), and compared it with two visual scorings from two research centres. In the second methodological chapter, we focused on the automatic detection of arousals in sleep EEG. We aimed to validate an automatic detection algorithm previously developed in the lab (Coppieters 't Wallant, Muto, et al., 2016). This algorithm was initially developed with the aim of identifying EEG segments comporting artefact and arousal events in order to remove them from further spectral analysis of EEG, and was validated as such. In this first article, we specifically determined its validity for arousal detection, by comparing it to arousal scoring of two experts from different research centres.

Though visual sleep scoring (whether stages or event detection such as arousals) remains the reference standard, in the era of big datasets, there is an increasing need for fast and replicable methods which allow comparison between studies and centres. Automatic algorithms, though sometimes approached with scepticism, present such advantages as they

score in a consistent, replicable manner and allow to get around the tedious and time-consuming task of visual scoring.

The remaining three chapters are found in the results section, and involve data collected at the Cyclotron Research Centre for the COFITAGE study (see next section for more details about the study). All three chapters aimed at investigating sleep characteristics that are associated with biomarkers identified as representing increased risk for future development of AD, and their link with cognition, whether at baseline or assessed longitudinally at 24 months.

In the first result chapter, we investigated sleep arousals, a microstructural element that has been previously examined mostly in the context of sleep pathologies. Spontaneous arousals, however, are an intrinsic part of sleep which have been reported to increase with age, while their mechanisms, correlates and functional consequences remain largely underinvestigated. We therefore assessed whether the potential heterogeneity of spontaneous sleep arousals was associated with A β burden in the sites known to show earliest aggregation, as well as with different aspects of cognition assessed during an extensive neuropsychological evaluation.

The second result chapter focuses on slow waves and spindles, two key waveforms of NREM sleep, and examines how their timely coupling, which has been previously described both as impacted in ageing, and relevant for sleep-related memory consolidation (Helfrich et al., 2018). In this section, we investigate the link between this precise co-occurrence of those two microstructural elements key to its sleep's mnemonic functions and early A β aggregates over the medial prefrontal cortex, and memory capacities, both as a baseline performance as well as its change over the two year longitudinal follow-up.

The third result section focused on the microstructural integrity of frontal regions, known to play a key role in sleep slow wave generation, and its link with slow wave characteristics. We examined the neurite density and complexity of its organisation in frontal grey matter using multi-shell diffusion-weighted imaging and neurite orientation dispersion and density imaging (NODDI), and related to EEG markers of neural synchronisation during sleep.

The main hypotheses behind those three result chapters presented in this work were that worse cerebral integrity, as quantified by poorer MRI markers and higher amyloid burden in otherwise healthy individuals would be associated to impaired sleep microstructure. We

also hypothesised that poorer sleep characteristics would be correlated with worse cognition assessed at baseline or predictive of the cognitive decline observed during the longitudinal follow-up.

Experimental protocol

The main outputs in the results section of this thesis come from data collected for the Cognitive fitness in ageing (COFITAGE) study, a large project encompassing a broad set of neuroscience techniques, all with the aim to identify new markers of ‘successful cognitive ageing’ (Daffner, 2010). This cross-sectional study was performed at the GIGA-Cyclotron Research Centre between June 2016 and October 2019, with the cognitive follow-up fully acquired only in October 2021. The general goal of this ambitious study was to characterise, in a relatively large sample of older individuals, sleep and wakefulness regulation processes, as well as reserve factors such as allostatic load, cognitive and brain reserve, and assess their relationship with well-established biomarkers of AD neuropathology.

The study enrolled 208 healthy older individuals aged 50-70 years, which were thoroughly screened for exclusion criteria, which were as follows: clinical signs of cognitive impairment (via the Dementia rating scale (≤ 130) and the Mini-Mental State Evaluation (≤ 27)); Body Mass Index (BMI) ≤ 18 and ≥ 29 ; recent psychiatric history or severe brain trauma; self-reported or clinically diagnosed sleep disorder; use of medication affecting sleep or the central nervous system; smoking; excessive alcohol (>14 units/week) or caffeine (>5 cups/day) consumption; shift work in the 6 months or transmeridian travel in the 2 months preceding the study. From the 208 recruited subjects, 101 met all inclusion criteria and constitute the final sample.

The whole protocol is represented in **figure 19**. Volunteers first came for a screening or recruitment session during which they filled questionnaires assessing their eligibility for the study. Subjects then came to the lab for an adaptation night, whose aim was double: to exclude volunteers presenting a sleep apnoea/hypopnea syndrome (subjects with an AHI ≥ 15 were rejected from further participation in the study) as well as to prevent so-called ‘first night effects’ during the proper sleep EEG recording (baseline night), as it was shown that sleeping in an unfamiliar environment for the first time might significantly impacts sleep metrics (Agnew, Webb, & Williams, 1966). A structural MRI at 3Tesla was performed, from which quantitative multi-parametric mappings and diffusion-weighted imaging were obtained. Two PET-scans were carried out, one with ^{18}F Flutemetamol (^{18}F Florbetapir for 3 subjects) and one with ^{18}F THK-5351 (in a subsample of 67 subjects) to assess amyloid-beta and tau protein burden/neurodegeneration, respectively. Two weeks of actimetry without fixed bed/wake-up schedule (free actimetry) were acquired, to gain an overview of each volunteer’s rest/activity habits in ecological settings. Then, a baseline night of sleep was recorded with EEG, following one week of tight-schedule actimetry with fixed bed and wake-up times based on each

volunteer's habits (fixed actimetry). Extensive neuropsychological assessment was carried out in two sessions while well-rested, with tests covering the attentional, memory and executive domains. The first sessions was performed between 12 and 6 hours before habitual bedtime, while the second one, administered on the day preceding the baseline night, took place ~7hours before habitual bedtime.

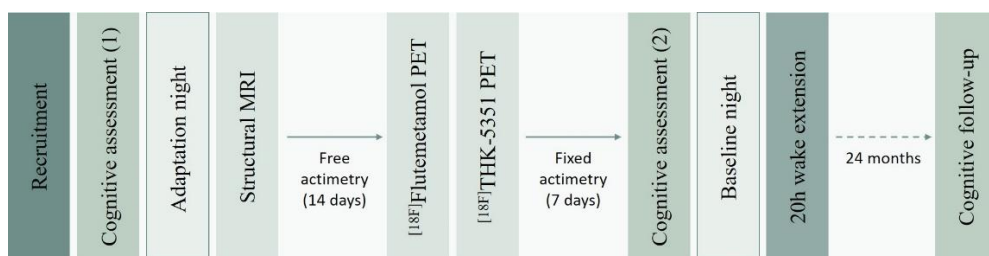


Figure 19. Overview of the COFITAGE study protocol. The adaptation night consisted in a polysomnography after which volunteers with sleep apnoea/hypopnea index $\geq 15/h$ were excluded. Blood samples were collected in the following morning, from which APOE status were extracted. Schedules for fixed actimetry and baseline night were adapted based on each volunteer's preferred bed and wake-up time. Cognitive assessments were performed in two sessions while well-rested. The order of the different steps varied between participants, except for the 7 days of fixed actimetry, cognitive assessment 2, baseline night and wake extension that were mandatorily kept in that predefined order.

Following the baseline night, a 20h wake extension protocol in constant routine conditions was performed, during which subjects were administered: 5 TMS-EEG sessions and resting-state wakefulness EEG recordings, and a battery of cognitive tasks were administered several times, along with salivary melatonin assays. The results of these aspects of the protocol can be found in the thesis of my colleague, Dr Maxime Van Egroo, as well as in a series of papers (Van Egroo et al., 2021; Van Egroo, Narbutas, Chylinski, Villar González, Ghaemmaghami, et al., 2019).

Additionally, a set of physiological measures was assessed, such as blood and urinary samples to compute allostatic load and genetic predisposition to AD, as well as lifestyle questionnaires to assess their role in the cognitive trajectory in ageing. The results concerning this part of the data can be found in the thesis of my colleague, Dr Justinas Narbutas, as well as in the papers he published on the subject (Narbutas, Chylinski, et al., 2021; Narbutas, Van Egroo, et al., 2021; Narbutas et al., 2019).

Chapter IV: Variability of sleep stage scoring in late midlife and early old age

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SUMMARY

Sleep stage scoring can lead to important inter-expert variability. Although likely, whether this issue is amplified in older populations, which show alterations of sleep electrophysiology, has not been thoroughly assessed. Algorithms for automatic sleep stage scoring may appear ideal to eliminate inter-expert variability. Yet, variability between human experts and algorithm sleep stage scoring in healthy older individuals has not been investigated. Here, we aimed to compare stage scoring of older individuals and hypothesized that variability, whether between experts or considering the algorithm, would be higher than usually reported in the literature. Twenty cognitively normal and healthy late midlife individuals' (61 ± 5 years; 10 women) night-time sleep recordings were scored by two experts from different research centres and one algorithm. We computed agreements for the entire night (percentage and Cohen's κ) and each sleep stage. Whole-night pairwise agreements were relatively low and ranged from 67% to 78% (κ , 0.54–0.67). Sensitivity across pairs of scorers proved lowest for stages N1 (8.2%–63.4%) and N3 (44.8%–99.3%). Significant differences between experts and/or algorithm were found for total sleep time, sleep efficiency, time spent in N1/N2/N3 and wake after sleep onset ($p \leq 0.005$), but not for sleep onset latency, rapid eye movement (REM) and slow-wave sleep (SWS) duration (N2 + N3). Our results confirm high inter-expert variability in healthy aging. Consensus appears good for REM and SWS, considered as a whole. It seems more difficult for N3, potentially because human raters adapt their interpretation according to overall changes in sleep characteristics. Although the algorithm does not substantially reduce variability, it would favour time-efficient standardization.

Keywords: aging, automatic scoring, electroencephalography, inter-expert variability

INTRODUCTION

Sleep stage scoring is a first, crucial step in analysing a sleep electroencephalogram (EEG) recording, whether in research setting or clinical practice. As it is partly subject to expert interpretation, it can carry substantial inter-expert variability. Although algorithm for automated scoring methods are becoming increasingly available, time-consuming human expert sleep stage scoring remains the reference method.

Previous studies on inter-expert agreement have reported average kappa values showing almost perfect agreement ($\kappa \sim 0.82$), after a prior training aiming at standardising the interpretation of the scoring rules in the same research centre (Whitney et al., 1998). Lower agreement is however reached when comparing scorers across different centres, with mean kappa values ranging from moderate ($\kappa = 0.57$) (Zhang et al., 2015) to good agreement ($\kappa = 0.72$ to 0.76) (Basner, Griefahn, & Penzel, 2008; Danker-Hopfe et al., 2009). Several factors may lead to lower inter-expert agreement, amongst which pathologies affecting sleep quality, such as periodic limb movements syndrome, obstructive sleep apnoea, but also depression, or neurodegenerative diseases such as Parkinson's disease (Danker-Hopfe et al., 2004). Agreement has also been reported to vary between sleep stages, with lower agreement in NREM 1 (N1) (Basner et al., 2008; Rosenberg & Van Hout, 2013) and slow-wave sleep (SWS) (Y. Do Kim, Kurachi, Horita, Matsuura, & Kamikawa, 2007; Monroe, 1969). Lastly, increasing the number of scorers being compared decreases the number of epochs upon which they all agree.

It is now well established that sleep changes across the lifespan, with regards to its architecture, but also its fine microstructure. These changes are detectable starting at age 40 (Carrier et al., 2011). Studies have reported that advancing in age is associated with an increased prevalence of N1 and N2 sleep stages, as well as a diminution of time spent in SWS and REM (Březinová, 1975; Ohayon et al., 2004; Redline et al., 2004). In addition, frequent awakenings may lead to more frequent N1 transitions, which can be strenuous to score. At a finer level, ageing has been related to a drop of EEG power and amplitude, particularly for the delta band (0.5-4 Hz) in anterior derivations (Gaudreau, Carrier, et al., 2001; H. P. Landolt & Borbély, 2001; Silber et al., 2007), and of the density (number per minutes of Non-Rapid-Eye-Movement – NREM - sleep) and amplitudes of detected slow-waves (Carrier et al., 2011). These changes are considered to arise from a decrease in the build-up of sleep need during wakefulness (Cajochen et al., 2006; Gaudreau, Morettini, et al., 2001) associated with a

decrease in the need for sleep (Klerman & Dijk, 2008) as well as a reduction in neurite density (Pannese, 2011) and/or cortical thickness (Dubé et al., 2015). A recent investigation showed that taking into account the reduced amplitude of sleep slow waves does not abolish age-related difference in SW density, and allows to include SW of lower amplitude that are seen in increased amounts in older individuals (T Rosinvil et al., 2020). The impact of age on inter-expert agreement remains scarcely investigated (Danker-Hopfe et al., 2009). One study which compared visual scoring in older individuals without using a fixed threshold for considering an oscillation as a slow wave showed good agreement in scoring for deep SWS (N3 and N4 sleep stages) (Webb & Dreblow, 1982). Yet, AASM recommends that scoring N3 stage should be based on the percentage of slow oscillations reaching at least a 75 μ V amplitude (Iber et al., 2007). Whether scoring agreement is good when applying (or trying to apply) AASM rules is not established (Berry et al., 2017).

The aim of this study was to assess inter-expert variability of visual and automatic sleep stage scoring in a population composed of healthy individuals in their late midlife/early old age, i.e. between 50 and 70 y when sleep is significantly altered by age, though potentially moderately. We compared scorings of three raters, two experts from different research centres, and an algorithm automatic sleep scoring that was previously validated in healthy young individuals and across several sleep disorders (Berthomier et al., 2007; L Peter-Derex et al., 2020). We hypothesised that given the changes in EEG density/amplitude associated with ageing, agreement would prove lower than what is usually reported in the literature for healthy young individuals, whether between experts or between experts and algorithm scoring, particularly over N3 stage.

METHODS

Dataset

Twenty sleep EEG recordings from healthy individuals in late midlife/early older age (61 ± 5 y; 10 women) were analysed in this study. Sample size was determined based on a prior sensitivity analysis which indicated that with a sample of $N = 20$, with a power of 0.8 and alpha of 0.05 in a multiple regression scheme, we were in a position to detect medium effect ($r > .28$; $R^2 > 0.078$) which we considered as likely prior to starting the study (as assessed using Gpower 3.1.9.4 (Faul, Erdfelder, Buchner, & Lang, 2009)). Exclusion criteria were: self-reported sleep or cognitive complaints, smoking, intake of medication affecting the sleep-wake

cycle or the central nervous system, chronic medical condition that may affect sleep (e.g. pain) as assessed via an in-house questionnaire and semi-structured interview, and shift work or transmeridian travel in the 3 months preceding participation in the study. An extensive expert neuropsychological assessment allowed to rule out any potential cognitive impairment. Before proper data acquisition, an adaptation and screening night under polysomnography was performed, and volunteers with apnoea/hypopnea index $>10/h$ or periodic limb movements of sleep associated with an arousal $>10/h$ were further excluded. All participants signed a written consent form and the study was approved by the Hospital of Sacré-Coeur de Montréal ethical committee.

Sleep recordings were acquired using a Grass Model 15A54 amplifier system. Sampling rate was set at 256 Hz, gain at 10.000, hardware filters were set at 0.3 Hz and 100 Hz for EEG, with a notch filter at 60 Hz. Twenty EEG derivations were placed according to the 10-20 system, referenced against the mean of the two mastoids, as well as submental electromyogram (EMG) and electrooculogram (EOG) bipolar channels.

Algorithm scoring (ALGO)

Aseega (PHYSIP, Paris, France) is an algorithm for automatic sleep scoring that relies on the analysis of a single EEG bipolar channel, either Cz-Pz or C4-O2, without further input from neither EMG nor EOG channels. The detailed procedure can be found in Berthomier et al., 2007. Sleep recordings were analysed twice independently, first using Cz-Pz and then C4-O2, thus providing two sets of automatic hypnograms. Cz-Pz results can be found in the results section, while C4-O2 analysis can be found in supplementary materials (boxplot of kappa and percentage of agreement in suppl. figure 1, contingency matrix in suppl. table 1, and agreement coefficient for comparisons with the algorithm (sensitivity, specificity, positive and negative predictive values (see statistical analysis) in suppl. table 2).

Expert scoring

Visual scoring was performed independently by two human sleep experts, EXPERT_1 and EXPERT_2, one at the CARSM in Montréal, Canada and one at the GIGA-CRC-IVI in Liège, Belgium, using AASM rules (Iber et al., 2007). No attempt for alignment was established prior the execution of the study. The expert consensus, EXPERT_CON, was defined as the set of epochs for which EXPERT_1 and EXPERT_2 agreed.

Statistical analysis

Scores were first compared on an epoch-by-epoch basis. Pairwise comparisons between EXPERT_1, EXPERT_2, EXPERT_CON and ALGO, for both Cz-Pz and C4-O2 analyses, were performed. Several agreement coefficients were computed: percentage of agreement, calculated as the number of epochs that were assigned the same sleep stage over the number of epochs for each recording; and Cohen's kappa (J. Cohen, 1960) (see Table 1 for Cohen's kappa values' interpretation according to (Landis & Koch, 1977)). Additionally for ALGO vs. EXPERT_CON, we computed the kappa value on pooled nights' scorings, i.e. obtained by concatenating all the nights together, in order to counter potential night length effect, i.e. without attributing more/less weight to short/long nights as it is the case when computing mean of kappa across separated night. (Berthomier et al., 2007). For each sleep stage, we also computed the following: sensitivity, defined as the number of epochs assigned a specific sleep stage by both ALGO and EXPERT_CON over the number of epochs scored as that stage by the EXPERT_CON; positive predictive value, PPV, calculated as the number of epochs assigned a specific sleep stage by both ALGO and EXPERT_CON over the number of epochs scored as that stage by the ALGO; specificity, defined as the number of epochs assigned as other than a specific stage by both scorers divided by the number of epochs assigned as other than that the specific stage by the rater used as reference; and negative predictive value (NPV), defined as the number of epochs assigned as other than a specific stage by both scorers over the number of epochs assigned any other sleep stage by the rater used as reference (Altman DG, 1997).

Finally, potential rater effects on sleep variables (sleep onset latency to the first N2 epoch, SOL; wake after sleep onset, WASO; total sleep time, TST; time spent in N1/N2/N3/REM, tN1/tN2/tN3/tREM; sleep efficiency, SE; number of stage shifts, NSS) were assessed through several generalised linear mixed models (GLMMs) with each sleep variable as a dependent variable in turn, using SAS version 9.4 (SAS Institute, Cary, NC). The distribution of dependent variables was determined by fitting all parametric probability distributions to data, using the "allfitdist" function in Matlab (<http://amir.eng.uci.edu/MvCAT.php>) (The Mathworks Inc., Natick, MA) and GLMMs were adapted accordingly. Subject was put as a random factor (intercept), and statistical significance was set at $p < .05$. Degrees of freedom were estimated using Kenward-Roger's correction and p-values in post-hoc contrasts (differences of least square means) were adjusted for multiple testing using Tukey's procedure.

RESULTS

EXPERT_1 versus EXPERT_2: whole-night agreements

We first considered the agreement between the two experts. Percentage of agreement ranged between 56% and 86%, with a mean of 76 % (Figure 1A). Kappa values oscillated between 0.41 and 0.82, with 0.67 on average (Figure 1B), which constitutes a substantial agreement.

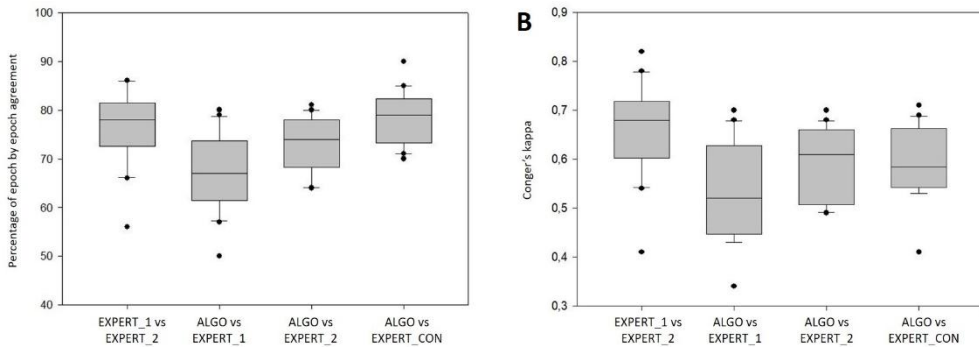


Figure 1. A. Boxplot of percentage of agreement between visual scorers (EXPERT_1 and EXPERT_2), algorithm scoring (ALGO) and EXPERT_1, ALGO and EXPERT_2 and ALGO and consensus of expert rater scorers (EXPERT_CON); B. boxplot of Cohen's kappa for the same comparisons. The boxes' central line represents mean values while one dot corresponds to one recording, and outliers were not removed from the plot.

ALGO versus EXPERTs: whole-night agreements

For the comparisons with ALGO, results were quite similar when using Cz-Pz or C4-O2. We detailed here the results on Cz-Pz. See suppl. for results using C4-O2.

Percentage of agreement between ALGO and EXPERT_1 ranged from 50 to 80% with a mean of 67%, and κ ranged from 0.34 to 0.70 (mean 0.54; moderate agreement) (Figure 1A-B). For ALGO versus EXPERT_2, agreement values reached 64 to 81%, with a mean of 74%, and κ values went from 0.49 to 0.70, with a mean of 0.60 (substantial agreement). After discarding the epochs of expert disagreement, percentage of agreement between ALGO and EXPERT_CON were highest and ranged from 70% to 90%, with a mean of 78%. Kappa values oscillated between 0.54 and 0.84, with a mean of 0.66 (substantial agreement; mean = 0.68 following the concatenation procedure for potential night length effect – see methods). The contingency matrix (Table 1), provided the sensitivity, specificity, PPV and NPV for each sleep stage (Table 2).

Table 1. Contingency matrix between the consensus of experts and algorithm scoring for the whole dataset.

Number of epochs (whole dataset)		Expert consensus (EXPERT_CON)						Total
		Artefact or disagreement	W	REM	N1	N2	N3	
Algorithm (ALGO)	Artefact	77	17	151	39	86	1	371
	W	655	1757	105	360	170	2	3049
	REM	651	45	2244	192	232	0	3364
	N1	160	35	96	142	113	1	547
	N2	2188	102	136	443	6162	516	9547
	N3	789	3	0	0	516	792	2100
	Tot	4520	1959	2732	1176	7279	1312	18978

Table 2. Sensitivity (Se), Specificity (Sp), Negative Predictive Value (NPV) and Positive Predictive Value (PPV) for agreement between scorers for each sleep stage.

		W	REM	N1	N2	N3
EXPERT_1 vs EXPERT_2	Se	74.2	84.8	63.4	73.3	99.3
	Sp	98.7	98.2	93.2	92.5	87.7
	PPV	89.9	90.8	50.3	91.5	37.6
	NPV	95.9	96.9	95.9	76.0	99.9
ALGO vs EXPERT_1	Se	83.9	83.9	8.2	83.1	44.8
	Sp	92.6	93.8	97.8	71.9	96.4
	PPV	59.8	71.0	34.2	68.3	74.1
	NPV	97.8	97.0	88.5	85.4	88.4
ALGO vs EXPERT_2	Se	85.1	85.0	11.6	80.0	60.4
	Sp	94.9	95.0	98.0	80.7	92.5
	PPV	73.2	77.1	38.0	82.2	38.0
	NPV	97.5	97.0	91.2	78.3	96.8
ALGO vs EXPERT_CON	Se	90.5	86.9	12.5	85.7	60.4
	Sp	94.8	96.0	98.1	82.8	96.0
	PPV	73.4	82.7	36.7	83.7	60.4
	NPV	98.4	97.1	92.8	84.8	96.0

EXPERT = human experts from 2 different research centres; ALGO = algorithm scoring; EXPERT_CON = expert consensus

Rater effect on sleep variables

GLMMs showed significant rater effects for all sleep variables (Figure 2 A-F, H-I) except SOL and tREM (Figure 2G) (Table 4). Post-hoc analyses showed that for tN1, experts did not significantly differ between themselves, but both differed from ALGO, with higher tN1 values than ALGO. For WASO, tN2 and tN3, EXPERT_1 differed from both EXPERT_2 and ALGO, with lower WASO and tN2 values, and higher tN3. TST and SE showed lower value for EXPERT_2 than for EXPERT_1, and lower for ALGO than for EXPERT_2. For the number of stage shifts, ALGO showed significantly lower values than the two experts, which showed no statistical difference for this variable. Interestingly, when pooling N2 and N3 duration together (i.e. SWS duration), no significant differences between scorers were detected (Table 3, Figure 3G). Figure 3 shows EXPERT_1, EXPERT_2 and ALGO scoring in a representative participant.

Table 3. Outcomes of GLMMs with each sleep variable assessing differences between rating methods.

	Main effect		Post-hoc					
			EXPERT_1 vs EXPERT_2		EXPERT_1 vs ALGO		EXPERT_2 vs ALGO	
	F(2,38)	p	t	p	t	P	t	p
SOL	0.97	.39	NA					
WASO	6.24	.0045	-3.32	.0055	-2.70	.0272	0.63	.8073
TST	21.36	<.0001	2.44	.0495	6.47	<.0001	4.03	.0007
tN1	40.28	<.0001	1.30	.4064	.34	<.0001	7.04	<.0001
tN2	28.21	<.0001	-7.06	<.0001	-5.75	<.0001	1.31	.3977
tN3	20.27	<.0001	6.29	<.0001	4.02	.0008	-2.26	.0738
tN2-N3	2.44	.10	NA					
tREM	1.97	.1540	NA					
SE	21.54	<.0001	2.51	.0426	6.50	<.0001	3.99	.0008
NSS	93.88	<.0001	-0.52	.8618	11.60	<.0001	12.12	<.0001

SOL = sleep onset latency; WASO = wake after sleep onset; TST = total sleep time; tN1/N2/N3/N2-N3/REM = time spent in N1/N2/N3/N2-N3 (without distinction)/REM; SE = sleep efficiency; NSS = number of stage shifts; ALGO = algorithm scoring; EXPERT = human experts from 2 different research centres

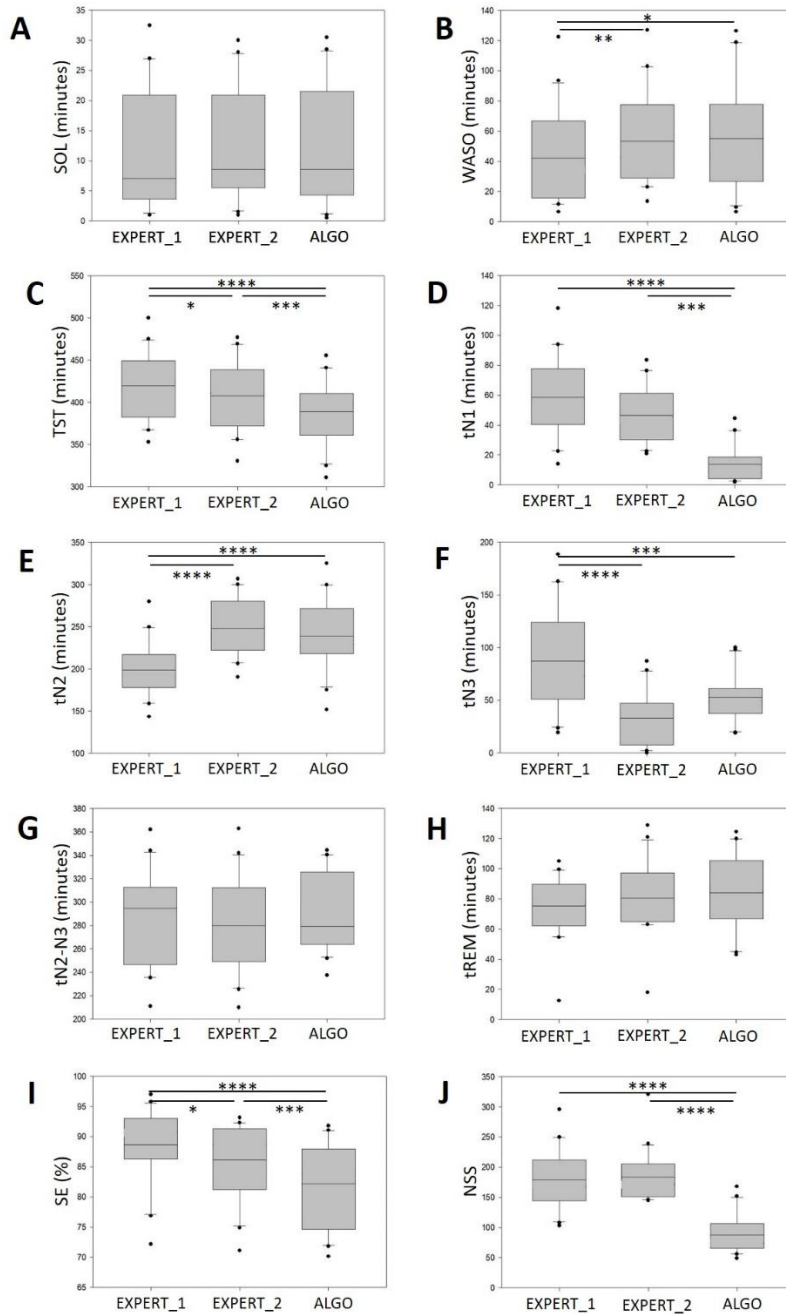


Figure 2. Boxplot of sleep variables values for each rating method (EXPERT_1, EXPERT_2 and ALGO). Panel A: sleep onset latency (SOL); B: wake after sleep onset (WASO); C: total sleep time (TST); D: time spent in NREM1 (tN1); E: time spent in NREM2 (tN2); F: time spent in NREM3 (tN3); G: time spent in NREM2 and NREM3 without distinction; H: time spent in REM (tREM); I: sleep

*efficiency (SE); J: number of stage shifts (NSS). The boxes' central line represents mean values, whiskers extend to the most extreme data points not considered outliers, and outliers were not removed from the plot. * means $p \leq 05$; ** means $p \leq 01$; *** means $p \leq 001$; **** means $p \leq 0001$*

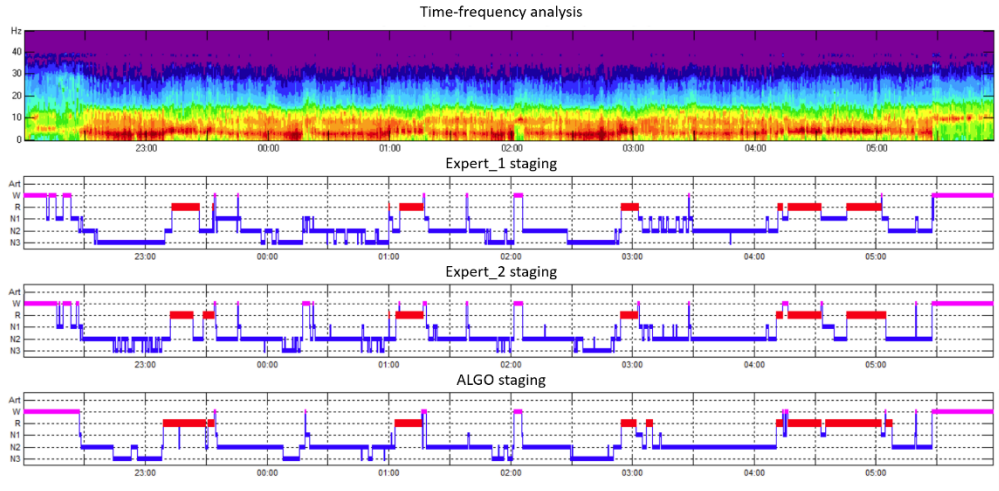


Figure 3. Representative example of scorings for each rater. From top to bottom: time frequency analysis, hypnograms of EXPERT_1, EXPERT_2, and algorithm automatic staging (ALGO). While the macrostructure seems identical, sleep is heavier in case of EXPERT_2 than for EXPERT_1, and ALGO seems to lay in between. At sleep onset, both experts put N1 while ALGO stays in W before going straight to N2.

DISCUSSION

Sleep stage scoring is of prime importance in sleep research as well as in sleep medicine. It remains mostly performed through visual inspection of the EEG. This leads to notable intra- and inter-expert variability when no alignment process is performed between experts (Berthomier et al., 2020). Inter-expert variability is likely to be higher when scoring sleep in individuals aged 40 or older, as their sleep, although normal and healthy, shows more frequent transitions between wake and sleep, and lower SW density and amplitude (Carrier et al., 2011; Gaudreau, Carrier, et al., 2001; Landolt & Borbély, 2001; Silber et al., 2007). Yet, investigation of this likely phenomenon remains insufficient. Hence, we investigated inter-expert variability at a relatively early stage of the ageing process, by comparing three sleep

stage scorings, two performed by visual experts from different centres (EXPERT_1 and EXPERT_2) without prior alignment between them, and one automatic scoring (ALGO) using a previously validated stage scoring algorithm (Berthomier et al., 2007; L Peter-Derex et al., 2020).

Our analyses show that agreement between the two experts was substantial ($\kappa=0.67$ on average), while it was lower, at moderate levels ($\kappa=0.54$ and 0.60), when comparing ALGO to the two experts, but substantial levels when ALGO was compared to the consensus of experts ($\kappa=0.66$; $\kappa=0.68$ for the concatenation of all recordings). These results fall into the lower range of previous Cohen's coefficient values for comparison between experts across different centres. Previous studies with prior expert alignment showed kappa values ranging from 0.57 to 0.78 in a mixed population (Basner et al., 2008; Danker-Hopfe et al., 2009; X. Zhang et al., 2015), or equal to 0.76 in a mainly healthy population (Danker-Hopfe et al., 2009) where the level of agreement was found to decrease with subjects age. Without prior expert alignment, kappa values ranged from 0.54 to 0.58 in a population mainly composed of patients (Zhang et al., 2015), or from 0.61 to 0.82 in various patient populations (Danker-Hopfe et al., 2004).

The main output of this study is the anticipated low agreement between the experts for N3 sleep. Agreement values showed that if nearly all the epochs scored as N3 by EXPERT_2 got the same label from EXPERT_1 (Se=99.3%), about only 1 out of 3 epochs of N3 epochs of EXPERT_1 were scored the same way by EXPERT_2 (PPV=37.6%). Regarding algorithm analysis, N3 assignment seemed to be intermediate between both experts. N3 scoring differences are illustrated in the representative subject displayed in figure 3. These differences may arise from the fact that N3 is a sleep stage heavily relying on an amplitude criterion ($75\mu V$), when amplitude is known to be affected by several factors, like skull or scalp thickness (Cuffin, 1993), or even the contact quality between the scalp and the EEG sensor. Furthermore, it is now well established that sleep undergoes a series of changes during ageing, starting as early as 40 y, amongst which a reduction in time spent in SWS and SW density (Carrier et al., 2011; H. P. Landolt, Dijk, Achermann, & Borbély, 1996; Mander et al., 2017). It was suggested that this reduction observed in ageing was due to an overall decrease in sleep EEG amplitude not specific to SW generation (Webb & Dreblow, 1982). Recent findings propose, however, that adapting the detection threshold decreases the differences seen between younger and older individuals, as it allows to pick up lower amplitude slow waves that are still generated in aged populations, but does not abolish them (T Rosinvil et al., 2020). Our findings

and these established variations in EEG signal call the 75 μ V criterion into question, particularly in the aged population.

The automatic scoring algorithm used here – Aseega - does not use absolute amplitude criteria, but rather relies on data-driven individual thresholds for scoring. Yet, it does not appear to assign more epoch to N3 than EXPERT_1. While visual scorers are relying on the AASM rules and should observe the 75 μ V criterion, in practice, scoring habits may emerge in sleep research centres, e.g. relying on other criteria like slow wave continuity or because of the typical population most recorded. Importantly when considering both N2 and N3 duration together, SWS as whole, staging methods do not differ anymore, further suggesting that it is the identification of lower amplitude oscillations as slow waves and whether they cover more than 20% of a 30s epoch that drives the differences between raters. This means that computation of SWS parameters, including slow wave activity (SWA), i.e. the EEG power within the delta band (0.5-4Hz) during NREM sleep, which is considered to reflect sleep need (Achermann, Dijk, Brunner, & Borbély, 1993), based on either of the experts or on ALGO would be similar. Since agreement was also high between raters for REM (see table 2, figure 2), computation of EEG power during REM (e.g. in the theta band – 4-8Hz) should also be similar across staging methods.

We also find low agreement for N1 sleep. The number of epochs scored N1 by any rater was low and significantly differed between experts, but it was even lower when using ALGO. This may not come as a surprise and might result from the low EEG characterisability of N1, as, apart from the occasional vertex sharp wave, stage N1 is mainly characterised by a reduction of alpha rhythm or activity in the theta range. It is thus a relatively unspecific stage, and ALGO might tend to prioritise classification in other stages. Indeed, 31% and 38% of epochs scored by both experts as N1 are scored as wakefulness and N2 by ALGO, respectively. This first suggests that the disagreement over N1 epochs happens in part at the beginning of the sleep opportunity, where ALGO tends to favour wakefulness longer and sleep onset directly to N2. The second may reflect the fact that ALGO better detects spindles embedded into a mixed frequency background. Importantly, only 16% of N1 epoch of experts are categorised as REM by ALGO. Notably, SOL, the time between light-off and the first N2 epoch, which is an important sleep variable including in clinical practice, was not significantly different across staging methods. Also, TST and SE differed between all three scorers.

Overall, we report non-negligible inter-expert variability on several classically used sleep variables when considering sleep recordings of individuals in their late midlife/early old age scored by experts from different centres, with no prior alignment process. Although beyond the scope of the current paper, since age-related changes are progressive, we suspect that this variability would be further exacerbated in even older individuals. This would however deserve further investigation. Inter-expert scoring variability across research centres does not imply that the effect of an experiment manipulation cannot be detected in older individuals within a research centre where sleep recordings are usually scored the same way (and often by the same experts). It speaks for caution, however, when comparing results across research centres (e.g. in BIG data studies), and may prevent from getting normative values from the literature. It also highlights the importance of scoring rules and scoring criteria for sleep stage scoring. Though sleep inarguably undergoes a series of changes with ageing, an important question remains to what extent the reported changes are influenced by the potentially considerable variability between sleep staging experts. It may be particularly important to regularly set a common ground to scoring, as recommended by the AASM, or to turn to automatic algorithms. The latter may not be better than human experts but will be systematic and consistent across and within research centres. Without such standardisation, it may prove difficult to compare scorings and thus extracted sleep variables, probably particularly so in aged populations where sleep is more fragile.

Chapter V: Validation of an automatic arousal detection algorithm for whole-night sleep EEG recordings

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ABSTRACT

Arousals during sleep are transient accelerations of the EEG signal considered to reflect sleep perturbations associated with poorer sleep quality. They are typically detected by visual inspection, which is time consuming, subjective, and prevents good comparability across scorers, studies and research centres. We developed a fully automatic algorithm which aims at detecting artefact and arousal events in whole-night EEG recordings, based on time-frequency analysis with adapted thresholds derived from individual data. We ran an automated detection of arousals over 35 sleep EEG recordings in healthy young and older individuals, and compared it against human visual detection from two research centres with the aim to evaluate the algorithm performance. Comparison across human scorers revealed a high variability in the number of detected arousals, which was always lower than the number detected automatically. Despite indexing more events, automatic detection showed high agreement with human detection as reflected by its correlation with human raters and very good Cohen's kappa values. Finally, sex of participants and sleep stage did not influence performance, while age may impact automatic detection, depending on the human rater considered as gold standard. We propose our freely available algorithm as a reliable, time-sparing alternative to visual detection of arousals.

Keywords: arousals; electroencephalography; automatic detection; sleep; artefacts

INTRODUCTION

Sleep is a complex phenomenon constituted of alternating stages that are characterised by inhomogeneous patterns of neuronal activity. Arousals during sleep consist in transient accelerations of the electroencephalogram (EEG) entwined with the structure of the sleep EEG (Halász, 1998). Arousals are physiological components of sleep microstructure, thought to ensure the reversibility of sleep (Halasz et al., 2004), that can be triggered by exogenous or endogenous stimuli. They are seen in increased amounts in some sleep pathologies, such as sleep apnoea where they are concomitant with hypoxic events (Malhotra & Jordan, 2016). Arousals can be triggered by noise and their density is enhanced in noisy environments (Griefahn, Bröde, Marks, & Basner, 2008; McGuire, Müller, Elmenhorst, & Basner, 2016; Rudzik et al., 2018). Spontaneous arousals (i.e. not associated with exogenous nor by detectable endogenous stimuli, such as hypoxia) are present in normal, non-pathological sleep, and have been reported to increase with age even though they are present at all ages (Bonnet & Arand, 2007; Boselli et al., 1998). Overall, arousals may lead to shallower sleep stages and contribute to more fragmented sleep.

Throughout the years, arousals have been defined in various ways, though the most widely accepted definition has been proposed by the American Sleep Disorders Association (ASDA) in 1992 (Bonnet et al., 1992) and maintained later in American Academy of Sleep Medicine (AASM) publications (Berry et al., 2017; Bond, 2014; Iber et al., 2007). They are described as abrupt and transient shifts in EEG frequencies that last at least 3 seconds and may include theta and alpha frequencies and frequencies greater than 16 Hz (but not spindles), and must be preceded by at least 10 seconds of stable sleep. Thus, a minimum of 10 seconds of stable sleep separates two distinct arousals. In Rapid Eye-Movement (REM) sleep, arousals must be accompanied by an increase in submental electromyogram (EMG) amplitude for at least 1 second. In general, arousal scoring cannot, however, be based solely on changes in submental EMG but must involve aforementioned changes in EEG frequency. Importantly, arousal detection can affect sleep staging. According to AASM rules (Iber et al., 2007), once detected, an arousal may imply that the next 30s window of sleep should be scored in another (lighter) sleep stage (e.g. N3 to N2/N1/wake, N2 to N1/wake, REM to N1/wake) unless the sleep features present in the next 30s window plead to maintain the current sleep stage (e.g. presence of rapid eye movements in REM, or of k-complexes/spindles in N2). In addition, researchers favour exclusion of artefacts and arousals from spectral decompositions of the sleep EEG signal in order to quantify artefact-free EEG power in different frequency bands.

Detecting arousals is therefore an important step in characterising sleep in clinical practice and in research settings. Nowadays, the detection of arousal events is commonly done by human visual inspection of the recordings. This method has the disadvantage of being time consuming and driven by subjective interpretation of an ‘abrupt shift in EEG’, which may vary considerably among individual raters. Indeed, as for sleep stages or spindle scoring (Silber et al., 2007; Wendt et al., 2015), arousal scoring is subject to intra-rater variability (as scorers would not necessarily detect events consistently if presented with the same recording twice (Bonnet et al., 2007)), as well as inter-rater variability. The literature shows that inter-rater variability differs from study to study. It may be hard to compare them given the heterogeneity in methodology or agreement coefficients used, but what stands out is that while inter-rater agreement can be good when comparing visual scorers from the same centre (intraclass correlation coefficient .84 (Loredo et al., 1999), .90 (Wong, Galster, Lau, Lutz, & Marcus, 2004), event by event agreement 90% (Thomas, 2003), Cohen’s kappa .71 (Crowell et al., 2002)), it proves lower when comparing arousal scoring across different centres (Drinnan, Murray, Griffiths, & Gibson, 1998), when scoring is based solely on EEG traces without autonomic responses (heart rate or airflow) (Thomas, 2003), or when considering only light sleep periods (Bonnet et al., 2007).

As for sleep staging, where the importance of automatic methods is continuously growing (Berthomier et al., 2020; Faust, Razaghi, Barika, Ciaccio, & Acharya, 2019), automatic arousal detection represents a way of getting around these difficulties and increases reproducibility. In the past years, several automated algorithms for arousal detection based on EEG scoring have been proposed (Agarwal, 2005; Alvarez-Estevéz & Fernández-Varela, 2019; Cho, Lee, Park, & Lee, 2005; De Carli et al., 2004; De Carli, Nobili, Gelcich, & Ferrillo, 1999; Ugur & Erdamar, 2019). However, most of them are based on some form of deep learning and require a training phase (Miller, Ward, & Bambos, 2018; Zabihi et al., 2018), use Independent Component Analyses (ICA) (Crespo-Garcia, Atienza, & Cantero, 2008) or spatial and temporal features and their implementation remains largely user-dependent, or they have not been validated using a comprehensive set of agreement measures. Usually, automatic detection methods used for transitory events (e.g. sleep spindles) yield a higher count of events than visual detection (Coppieters ’t Wallant, Maquet, & Phillips, 2016; Warby et al., 2015).

Here, we adapted a validated and published automatic artefacts and arousals detection algorithm (Coppieters ’t Wallant, Muto, et al., 2016) which was developed as a method to detect the clean segments in EEG data in order to subsequently perform spectral power

analysis. We aimed to separate its detection into both artefacts and arousals, to offer a quantification tool for finer gradient analysis of arousals, which are meaningful events of the sleep EEG. A key characteristic of the algorithm is that detection thresholds are self-adjusting to individual recording features. We validated the arousals detection on a dataset composed of 35 undisturbed young ($N = 18$; age 24 ± 3 y.) and older ($N = 17$; age 61 ± 6 y.) participants' night sleep EEG recordings. They were first visually scored for sleep stages and arousals by 4 sleep expert human raters (HR) from Basel, Switzerland, and considered as a single rater (indicated through the manuscript as BAS). Arousals were then visually scored by another expert from Liège, Belgium (here defined as DC, first author) which had access to partial information about sleep stages (wake - NREM sleep - REM sleep) to avoid potential bias arising from sleep stage changes. Arousals were also automatically detected (AD). We computed several agreement coefficients: inter-rater agreement (S), Cohen's kappa (κ), sensitivity (Se), mean overlap of detected events (C) and false discovery ratio (FDR) considering different references, or gold standard detection (see methods, section 4.4.1 for coefficient definitions and computation). We further explored whether sex, age and sleep stage influence detection reliability. Our objective was to demonstrate that the algorithm is a reliable arousal detection method which gives similar agreement performances as human raters among themselves. Moreover, we expected more arousals to be detected through AD based on previous automatic detection methods for transitory events and that supplemental arousals would contain lower frequency oscillation – rendering them less obvious to the human eye.

MATERIALS AND METHODS

Dataset

This is a retrospective study, taking advantage of data collected and published elsewhere (Rudzik et al., 2018). Our dataset consisted of 35 whole night multichannel EEG recordings of undisturbed sleep in younger (age range: 19-29, $\mu = 24.07 \pm 3$, $N = 18$, 7 females) and older (age range: 51-70, $\mu = 61.38 \pm 6$, $N = 17$, 8 females) participants, randomly selected to have matching group sizes. Participants had good self-reported sleep quality, as assessed by the Pittsburgh Sleep Quality Questionnaire (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) ($PSQI \leq 5$), normal levels of daytime sleepiness, as assessed by the Epworth Sleepiness Scale (Johns, 1991) ($ESS \leq 10$), and did not present periodic limb movement disorder nor sleep-disordered breathing, as verified during a polysomnography prior to inclusion in the

study. The recordings contained 12 EEG derivations placed according to the 10-20 system (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1, Oz, O2), referenced against the average of the 2 mastoids, as well as submental electromyogram (EMG) and electrooculogram (EOG) bipolar channels, all collected via a Vitaport-3 digital recorder (TEMEC Instruments B.V., Kerkrade, The Netherlands); acquisition sampling rate: 256 Hz; storage sampling rate: 128 Hz. Signals were filtered at acquisition (between 0.159 and 30 Hz for the EEG/EOG and between 1 and 70 Hz for EMG).

Human arousals scoring

Recordings were first randomly assigned to one of four human raters (HR) in Basel (BAS1-4; Table 1 shows the distribution across raters) and scored for sleep stages and arousals according to standard criteria (Richard B. Berry et al., 2015). Importantly, BAS scorers were not blind to recordings' age group. The recordings were further visually scored for arousals by a second HR (DC) which had access to sleep staging information only in the form of NREM/REM/wake epochs, to avoid biasing that would incline to detect arousals in the case of a sleep change to a lighter NREM sleep stage, and was blind to participants' age group. A supplementary analysis with Kruskal-Wallis test for unequal samples showed no significant differences in Cohen's κ values between the different BAS scorers when using DC as gold standard (Chi square = 2.51, $p = .47$, $df = 3$), nor when using BAS as gold standard (Chi square = 3.36, $p = .34$, $df = 3$). BAS scorers were therefore all considered as one scorer for the remaining analyses.

Table 1. Distribution of recordings in the dataset across raters from Basel according to age and sex.

SCORER	YOUNG			OLDER			
	F	M	Total	F	M	Total	
BAS1	3	3	6	4	5	9	15
BAS2	0	4	4	2	2	4	8
BAS3	2	2	4	0	1	1	5
BAS4	2	2	4	2	1	3	7
TOTAL	7	11	18	8	9	17	35

Automatic arousals detection algorithm

For the automatic detection procedure, we adapted an algorithm initially developed for artefact detection (i.e. both true artefacts and arousals) which is based on time and frequency analysis with adapted thresholds derived from the data (D Coppieters 't Wallant et al., 2016). The method consists in successively applying several modules, which are briefly described hereafter. Modifications done to the original code consisted only in organisation and extraction of arousal events. More detailed information about signal processing can be found in (D Coppieters 't Wallant et al., 2016).

Preprocessing

Recordings are first filtered (Butterworth filter of order 3; high pass/low pass at 0.5/30 Hz for EEG; 0.1/5 Hz for EOG; 10/100 Hz for EMG), and considered in scoring windows of 30s, then partitioned into 1s epochs. The signal of each channel is further mean corrected.

Bad channel detection

EEG. Obvious bad channels, i.e. flat and noisy channels with a signal standard deviation (SD) lower than $1 \mu\text{V}$ or higher than $6.10^3 \mu\text{V}$, respectively, are detected over the entire recording. Then, a finer detection is performed over each 30s window using each channel's SD and comparing it to the SD of other (good) channels: a channel with a deviating ratio > 5 is marked as a bad EEG channel.

EMG. As EMG signal is typically displayed in a bipolar montage but can be inconsistently artefacted solely on one of the two channels, the module attempts to reconstruct usable data from the available EMG channels. Flat channels are detected per 30s window as EMG channels referenced to the montage reference displaying median value $< 0.1 \mu\text{V}$. If no channel is considered flat, noisy channel detection is performed per 30s window by identifying referenced channels for which the median is at least twice that of the other. Noisy channels are then tested to differentiate constant from transitory noise considering all 1s epochs in a given 30s window: a channel is noisy over a 1s epoch if the ratio of absolute mean values of both EMG channels is larger than 2, and noisy on a whole scoring window if more than half of the epochs ($> 15\text{s}$) are noisy. A composite EMG channel is finally reconstructed based on the remaining (clean) EMG signal arising from both or only one EMG channel(s).

Features extraction

Shift in EMG. Transient increases in muscular tone frequency/magnitude are detected in three steps. First, rejection of the highest abnormal activities is performed along the whole EMG channel, by identifying any recording period that reaches higher values than baseline EMG values extracted from the first four minutes of the recording, which are considered to be the highest muscular tone amplitude without artefact. A second step then detects peaks in EMG activity in shorter time windows in order to account for EMG background activity, by defining, for each scoring window, a specific threshold as being the output of a median filter applied on a symmetric-centred 3s-scoring window. A last step aims at identifying the relevant EMG peaks that could influence EEG, by assessing their intensity and duration and comparing them to an adapting threshold computed based on the first ten 1s epochs on both sides of the muscle tone peak.

Shift in EEG. In this step, two tests are performed over three frequency bands: alpha (7-13 Hz), beta (16-30 Hz) and theta (3-7 Hz). First, detection of abnormal EEG activity is carried out using a fixed threshold consisting in the median value of the power in the given frequency band (α , β , θ) of the whole recording. A second step takes into account the specific background EEG activity of a shorter time window. For each scoring window, all 1s epochs without a corresponding EMG shift are selected, plus the first ten 1s epochs without an EMG shift each side of that scoring window. For the three examined frequency bands, all shifts in EEG higher than the adapted threshold corresponding to twice the median value of the selected epochs are considered. 1s epochs considered as containing a spindle are computed as those which relative power in the sigma band i.e. 11-16 Hz (ratio of the sigma band power over the sum of power in alpha, sigma and beta bands) is higher than 85% of the maximum relative power in the sigma band over the entire recording.

Arousal detection

Finally, shifts in EEG in the α , β , or θ frequency band (but excluding those concomitant with a shift in the sigma band) lasting over at least 3 consecutive 1s epochs are selected. These are further checked for muscular tone increase to be classified as EMG-associated events. In REM, only events with a concomitant EMG tone increase are considered as arousals.

Comparison between raters

As automatic detection (AD) reports events in seconds, HR scores were converted into 1s resolution: any 1s epoch containing more than 0.5s marked as arousal was labelled as containing an arousal. To compute agreement coefficients, one must compare the detection of a rater against another that is considered as the gold standard. For comparison between HR, as there is no way to know which events are “true arousals”, detection of each HR was compared against the detection of the other HR in turn. To compare AD to HR, 2 HR detections were created: an inclusive detection (HR inclusive), with all arousals found by HR (no matter if they were identified only by one HR, or by both), and a conservative detection (HR conservative), with only those arousals that were identified by both HR. As it is possible that the detection of a frequency shift in EEG as an arousals is more likely in the presence of a concomitant EMG tone increase, analyses considered all arousals found by the AD, as well as those found by AD that were associated with an EMG tone increase (AD EMG).

Two types of comparison were made with HR as gold standard: at the level of a 1s epoch and at the event level, i.e. the set of consecutive 1s epochs forming an arousal, where events were considered as common between HR and AD if there was at least 1s of the event overlapping across raters. Each comparison receives one of the following labels (Figure 1):

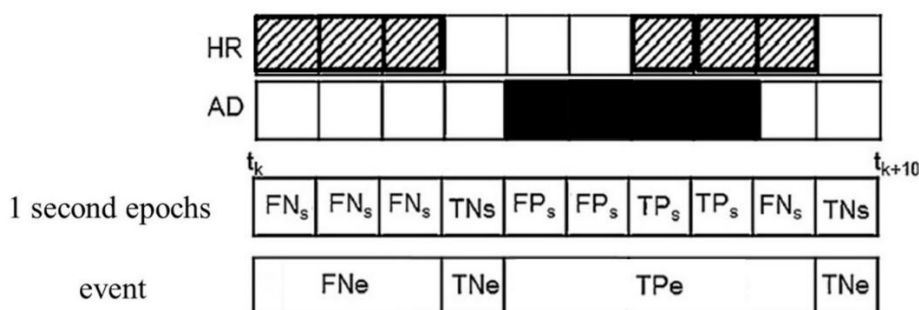


Figure 1. Two types of comparison level made: the 1s epoch or event. Gold standard human rater (HR) scoring is represented on the top line, with arousals marked by the hatched squares; Automatic Detection (AD) is on the second line, with black squares marking detected events. Adapted from (D Coppieters 't Wallant et al., 2016)

- True positive (TP): 1s epoch/event marked as arousal both by AD and HR
- False positive (FP): 1s epoch/event marked as arousal by AD but not by HR
- True negative (TN): 1s epoch/event marked as arousal-free by both AD and HR
- False negative (FN): 1s epoch/event marked as arousal free by AD but as an arousal by HR

Statistical parameters

We computed agreement with several coefficients classically found in the literature. At the 1s epoch level, we computed two values, the inter-rater agreement S (Bennett et al., 1954) and Cohen's Kappa (κ) (Cohen, 1960) following the equations below. Although inter-rater agreement S does not account for the unbalanced nature of the data to compare (the ratio between epochs containing arousals and those who don't is usually around 5 to 95%) and tends thus to overestimate inter-rater concordance, it is amongst the most commonly used. Cohen's Kappa is, in contrast, taking into account the unbalanced scores but is frequently judged overly conservative. Interpretation of κ values can be seen in Table 2 (from (Landis & Koch, 1977)).

$$S = 2 \times P_0 - 1 \quad \text{where} \quad P_0 = \frac{TP + TN}{TP + TN + FP + FN}$$

$$\kappa = \frac{P_0 - P_r}{1 - P_r} \quad \text{where} \quad P_r = \frac{(TP + TN) \times (TP + FP)}{(TP + TN + FP + FN)^2}$$

Table 2. Cohen's κ values and their interpretation, from (Landis & Koch, 1977)

Kappa value	Interpretation
< .00	Poor
.00 - .20	Slight
.21 - .40	Fair
.41 - .60	Moderate
.61 - .80	Substantial
.81 - 1.00	Almost perfect

At the event level, we calculated inter-rater agreement via the 3 following: sensitivity in terms of the percentage of detected event (Se), averaged overlap of the detected events (C) and false discovery ratio (FDR). Sensitivity gives an idea of how well the AD detects events that are detected by the gold standard; C is the mean proportion of TP_s contained in TP_e (delimited by the HR's detection); FDR corresponds to the proportion of false detections to the total number of detections.

Time frequency analysis

As AD detected many more arousals than HR, we wanted to further characterise the events that were not visually detected. For this analysis we took into account only the first three seconds of each arousal, considering that by definition an arousal lasts minimum 3 seconds. We performed a time-frequency analysis of all AD arousals using Morlet's method (Tallon-Baudry & Bertrand, 1999) with 1 Hz bins, after performing a baseline correction on the first 500ms. We summed the power in each 1 Hz bin for the 3s duration, and computed their relative power by dividing the power in each bin over the power in all frequency bins (0.5 – 29.5 Hz). We then computed the sum of the relative power in the theta (4.5 – 7.5 Hz), alpha (8.5 – 11.5 Hz) and beta (16.5 – 29.5 Hz) bands.

Statistical analyses

Statistical analyses were performed in SAS 9.4 (SAS Institute, Cary, NC). For generalised linear mixed models (GLMM), distribution of dependent variables was first determined in MATLAB using the allfitdist function (developed by Mike Sheppard), and models were appropriately adjusted. Subject was put as a random factor. Statistical significance was set at $p < .05$. 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 using Tukey's procedure.

RESULTS

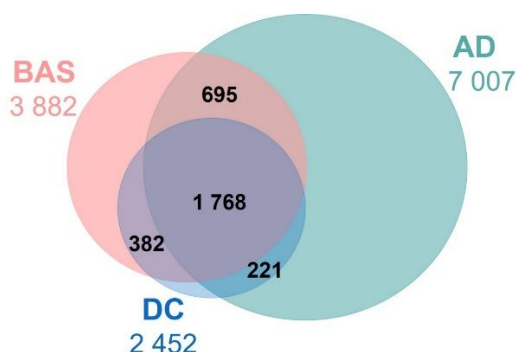


Figure 2. Total number of detected arousal events over all 35 recordings for Basel HR (BAS), Liège HR (DC), and automatic detection (AD) as well as their overlap.

Comparison of human raters (HR)

Comparing detections can only be done if a "ground truth" or suitable approximation (i.e., an expert HR as gold standard) is used as reference. As a first step, visual arousals detection were compared across HR, using each HR as gold standard in two separate analyses (Table 3). Agreement was high in both analyses, with inter-rater agreement S and Cohen's κ values indicating very good agreement (see Table 2 in section 2.4.1. for Cohen's kappa values and interpretations, from (Landis & Koch, 1977)), so that we considered both detections as good. BAS detected more arousals than DC (see Figure 2) which was reflected by a relatively low sensitivity when using BAS as gold standard and a high FDR when using DC as gold standard. In general, arousals detected by DC were also detected by BAS as reflected by a low FDR when using BAS as gold standard and a high sensitivity when using DC as gold standard. Commonly detected arousal events were well agreed between raters with about 75% of 1s epoch overlap for each detected event (C).

Table 3. Agreement coefficients (mean and standard deviation) between HR, with each HR being compared in turn to the other considered as gold standard: S_s (inter-rater agreement); κ (Cohen's kappa); S_e (sensitivity); C_s (mean overlap of events); FDR_e (false discovery ratio).

Gold standard	Compared	S_s	κ_s	S_e	C_s	FDR_e
BAS	DC	94 ± 3 %	0.97 ± 0.02	58 ± 16 %	72 ± 7 %	36 ± 12 %
DC	BAS	89 ± 4 %	0.94 ± 0.02	81 ± 26 %	78 ± 12 %	78 ± 9 %

Automatic arousal detection (AD) vs human raters

We then computed agreement coefficients for AD by comparing it against two gold standard HR detections: either including all the arousals found by either of the HR (HR inclusive) or only those common to both HR (HR conservative) (Table 4). Agreement was high, with κ values indicating very good agreement, and inter-rater agreement S was very good (> 85%), when using both HR inclusive and HR conservative as gold standard (Figure 3). Similarly to HR comparison, commonly detected arousal events were well agreed between raters with about 60% of 1s epoch overlap for both HR inclusive and HR conservative as gold standard. As expected, AD detected many more arousals than HR (cf. Figure 2; 1.8 times more than BAS and 2.9 times than DC) with high FDR values, particularly for HR conservative,

which is to be expected as this detection comprises substantially less events. Considering only AD arousals associated with a concomittant EMG tone increase substantially improves FDR, with little impact on indices S_s , κ and C_s , but, expectedly, reduces Se .

Table 4. Agreement coefficients (mean $\pm$ standard deviation) for all recordings between AD and HR scoring, for both HR inclusive and HR conservative detection as gold standard. EMG indicates automatically detected events that are accompanied by a submental EMG tone increase.

Gold standard	S_s	κ_s	Se_e	C_s	FDR_e
HR inclusive	$86 \pm 6 \%$	0.93 ± 0.03	$67 \pm 23 \%$	$59 \pm 13 \%$	$61 \pm 16 \%$
EMG	$90 \pm 6 \%$	0.95 ± 0.03	$45 \pm 23 \%$	$61 \pm 15 \%$	$28 \pm 25 \%$
HR conservative	$88 \pm 4 \%$	0.94 ± 0.02	$83 \pm 26 \%$	$58 \pm 14 \%$	$74 \pm 12 \%$
EMG	$92 \pm 4 \%$	0.96 ± 0.02	$64 \pm 27 \%$	$60 \pm 15 \%$	$41 \pm 23 \%$

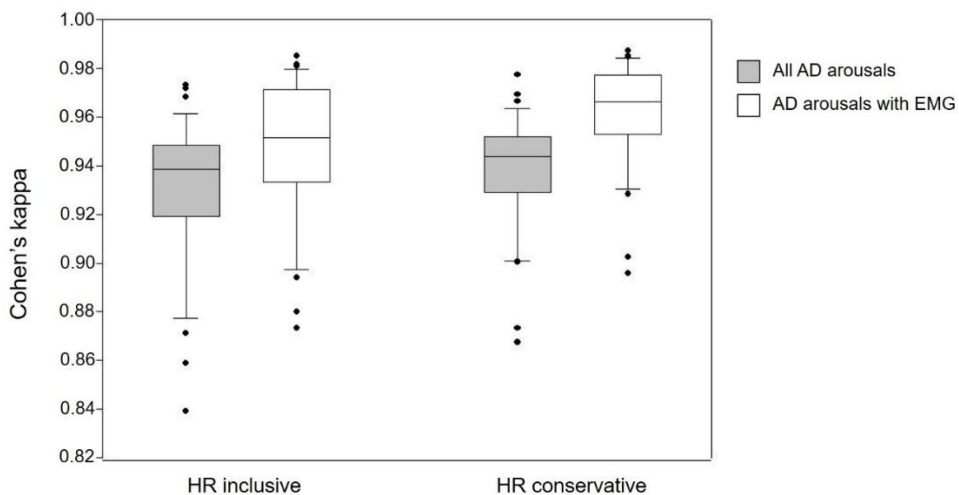


Figure 3. Box plot of Cohen's kappa values for HR inclusive and HR conservative as gold standard (values for all AD arousals in grey, for EMG-associated AD only in white). The boxes' central line indicates the median of κ values, with the bottom and upper adges showing the 25th and 75th percentiles, respectively. The whiskers extend to the most extreme data points not considered outliers; outliers were not removed from the plot.

Impact of age and sex

We checked whether recordings of older individuals bear more arousals than recordings of younger individuals. Two sample t-tests revealed no such significant difference, except for Basel scoring ($t(1,25.11) = -3.95$, $p = .0006$) (Table 5).

Table 5. Raw number of arousals detected per rater for young and older individuals (mean $\pm$ SD)

	DC	BAS*	Inclusive	Conservative	AD	AD EMG
Young	63 $\pm$ 31	84 $\pm$ 30	93 $\pm$ 37	51 $\pm$ 19	208 $\pm$ 48	68 $\pm$ 22
Old	79 $\pm$ 34	141 $\pm$ 53	142 $\pm$ 46	67 $\pm$ 23	193 $\pm$ 39	70 $\pm$ 25

* significant difference between age groups ($p = 0.0006$)

Generalised Linear Mixed Models (GLMMs) with each of the agreement coefficient as the dependent variable in turn, showed that for AD against HR inclusive, there was a significant effect of age on sensitivity and FDR, with higher values for both in the young group (Table 6). For events automatically detected with a concomitant submental EMG tone increase against HR inclusive as gold standard, significantly higher values were found in the younger age group for inter-rater agreement, Cohen's κ and sensitivity.

When comparing AD against HR conservative, no significant effect of age nor sex was found. When taking into account only AD events with concomitant EMG tone increase higher sensitivity for the younger age group was found, as well as a slightly higher inter-rater agreement and Cohen's κ for female recordings.

Given the high number of tests performed, a corrected p value threshold was set at $p = .005$ (Bonferroni correction for 10 comparisons; for each HR detection - inclusive/conservative - 5 coefficients $\times$ 2 comparison levels - all AD and AD EMG). Only inter-rater agreement S and Cohen's κ for HR inclusive, when taking into account AD EMG events, meet the correction criteria.

Table 6. Results of GLMMs for each agreement coefficient and age/sex for Automatic Detection (AD) against HR inclusive and conservative detection as gold standard, with all AD events (normal) and only AD EMG-associated events (italic).

Gold standard	AD arousals		S _s	κ _s	Se _e	C _s	FDR _e
HR inclusive	All	Age	p = .10	p = .12	p = .01	p = .24	p = .05
			F = 2.83	F = 2.49	F = 7.53	F = 1.42	F = 4.23
		Sex	p = .10	p = .10	p = .16	p = .21	p = .49
			F = 2.91	F = 2.83	F = 2.12	F = 1.63	F = .48
	<i>EMG</i>	<i>Age</i>	<i>p = .002*</i>	<i>p = .002*</i>	<i>p = .01</i>	<i>p = .41</i>	<i>p = .61</i>
			<i>F = 11.48</i>	<i>F = 11.03</i>	<i>F = 6.89</i>	<i>F = 0.71</i>	<i>F = .26</i>
		<i>Sex</i>	<i>p = .07</i>	<i>p = .07</i>	<i>p = .78</i>	<i>p = .13</i>	<i>p = .70</i>
			<i>F = 3.49</i>	<i>F = 3.48</i>	<i>F = .08</i>	<i>F = 2.41</i>	<i>F = .15</i>
HR conservative	All	Age	p = .96	p = .91	p = .33	p = .86	p = .07
			F = .00	F = .01	F = .99	F = .03	F = 3.52
		Sex	p = .21	p = .21	p = .12	p = .27	p = .37
			F = 1.64	F = 1.62	F = 2.49	F = 1.24	F = .82
	<i>EMG</i>	<i>Age</i>	<i>p = .09</i>	<i>p = .10</i>	<i>p = .05</i>	<i>p = .97</i>	<i>p = .88</i>
			<i>F = 3.05</i>	<i>F = 2.87</i>	<i>F = 4.21</i>	<i>F = .00</i>	<i>F = .02</i>
		<i>Sex</i>	<i>p = .04</i>	<i>p = .04</i>	<i>p = .45</i>	<i>p = .21</i>	<i>p = .56</i>
			<i>F = 4.68</i>	<i>F = 4.71</i>	<i>F = .58</i>	<i>F = 1.63</i>	<i>F = .35</i>

All F tests had 1 (main effect) and 32 (error) degrees of freedom

* significant after correcting for multiple comparisons ($p < 0.005$ – Bonferroni corrected)

In summary, while sex of the recorded participant did not seem to influence the agreement between AD and HR, age may impact agreement particularly when considering HR inclusive as gold standard and when considering only EMG-associated AD events.

Impact of sleep stage

As not all types of events (true positive, true negative, false positive and false negative) were present for all sleep stages, comparison coefficients based on events (rather than seconds) were not suitable for testing differences in agreement across sleep stages (see methods, section 2.4 for coefficient computation). Hence, arousal detection over different sleep stages was compared only using comparison coefficients based on 1s epochs (i.e. Cohen's κ and inter-rater agreement S). For comparison of AD against HR inclusive, a GLMM with Cohen's κ as dependent variable showed no significant effect of age ($F(1,31.43) = 2.24$, $p = .15$), nor of sex ($F(1,31.53) = 2.91$, $p = .10$) nor sleep stage ($F(3,72.55) = .42$, $p = .74$). Likewise, another GLMM with inter-rater agreement S showed no significant effect of any of the three variables (age $F(1,1.59) = 2.55$, $p = .12$; sex $F(1,31.68) = 3.01$, $p = .09$; sleep stage $F(3,72.82) = .43$, $p = .73$). Another set of GLMMs of AD against HR conservative showed no significant effect of age ($F(1,31.69) = .02$, $p = .88$), sex ($F(1,31.77) = 1.66$, $p = .21$), nor sleep stage ($F(3,72.29) = .08$, $p = .97$). As for inter-rater agreement, S, again, no significant effect of age ($F(1,31.77) = .01$, $p = .93$), sex ($F(1,31.84) = 1.7$, $p = .20$) or sleep stage ($F(3,72.53) = .09$, $p = .97$) was found. Figure 4 shows the distribution of Cohen's κ values across sleep stages for HR inclusive and conservative as gold standard.

These results indicate that when taking into account the potential variability in AD performance according to sleep stages, agreement between AD and HR does not depend on age, sex or sleep stage.

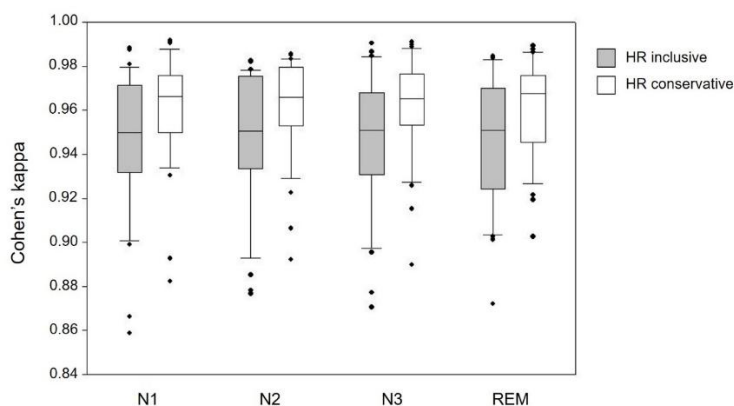


Figure 4. Box plot of Cohen's kappa values for HR inclusive and conservative detection as gold standard by sleep stage. The boxes' central line indicates the median of κ values, with the bottom and

upper adges showing the 25th and 75th percentiles, respectively. The whiskers extend to the most extreme data points not considered outliers, and outliers were not removed from the plot.

Correlation between AD and HR

A next question we asked beyond these agreement estimations was whether AD and HR were correlated, i.e. whether variation in arousal density across recordings were similarly detected by HR and AD. Pearson r indicated that the correlation between AD and HR inclusive was $r = .30$ ($p = .08$) and $r = .23$ ($p = .19$) for all AD events (Figure 5, panel A) and AD EMG-associated events, respectively. For HR conservative, correlation was $r = .38$ ($p = .025$) and $.36$ ($p = .037$) for all AD events (Figure 5, panel B) and AD EMG-associated events, respectively. It seems therefore that AD is best associated with the most conservative detection of arousals across centres.

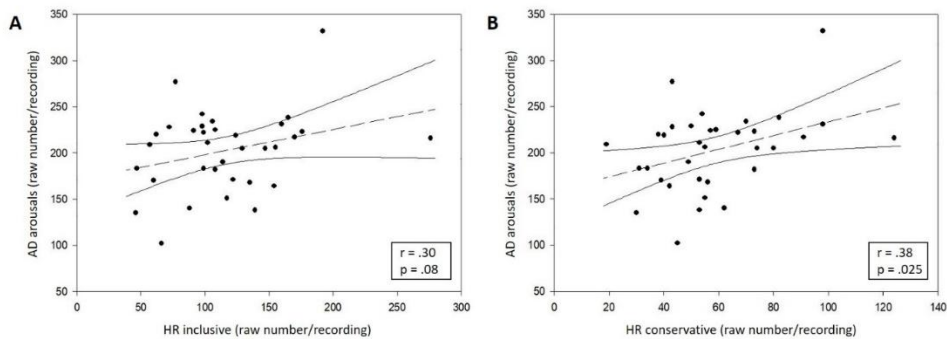


Figure 5. Correlation between all AD arousals and HR, for inclusive (A) and conservative detection (B)

Characterisation of the arousals only detected by AD

As AD detected many more arousals than HR, we wanted to further characterise the events that were not visually detected by any HR but were still picked up by AD, to see if they differed in terms of frequency or power. To do so, we performed a time-frequency analysis on the first 3 seconds of all AD arousals, in the frequencies 0.5 Hz-29.5 Hz by 1 Hz bins. We summed over time the power in each 1 Hz bin, and normalised it by dividing by the in all frequency bins to obtain relative power. We then computed the sum of the relative power in the theta (4.5 – 7.5 Hz), alpha (8.5 – 11.5 Hz) and beta (16.5 – 29.5 Hz) bands.

A GLMM with relative power as dependent variable showed a main effect of frequency band ($F(2,21101) = 9991.38$, $p < .0001$), of rater ($F(1,21101) = 296.47$, $p < .0001$) and an interaction between frequency band and rater ($F(2,21101) = 473.23$, $p < .0001$). Post hoc analysis showed the theta band had a significantly lower power ($p < .0001$), while the alpha and beta a significantly higher power ($p < .0001$) for AD arousals that were also detected by an HR. Figure 6 shows mean relative frequency for the theta, alpha and beta band for arousals that were detected only by AD and those detected by AD and HR.

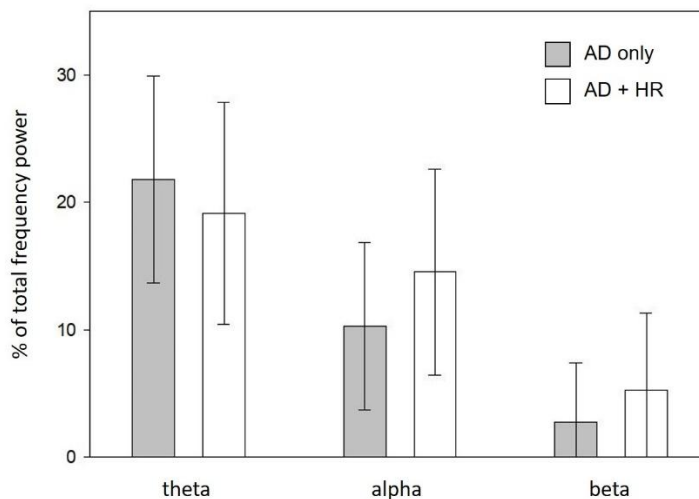


Figure 6. Mean relative frequency power for the theta, alpha and beta band for arousals detected only by AD (grey) and those detected by AD and HR (white). Error bars show standard deviation.

DISCUSSION

Arousals are transient changes in neuronal activity that are part of normal sleep microstructure and are reflected in temporary acceleration of the EEG (Bonnet et al., 1992). They are either spontaneous and show an increased prevalence with age, or triggered by internal or external events (Richard et al., 2015). Their detection is important for sleep stage determination and spectral composition analyses as well as pathology characterisations, as they are considered to reflect poorer sleep quality. Here, we validate an automatic detection (AD) of spontaneous arousals using non-pathological sleep EEG recordings and human visual detection in two research centres as reference (Centre for Chronobiology in Basel, Switzerland, and GIGA-Cyclotron Research Centre-In Vivo Imaging in Liège, Belgium). AD was adapted

based on a previously validated tool for the undifferentiated detection of both artefacts and arousals (Coppieters 't Wallant, Muto, et al., 2016). Our analyses first confirm that the number of spontaneous arousals visually detected by human raters (HR) can vary a lot, with sensitivity and FDR that can be, respectively, lower and higher depending on which rater was considered as the gold standard (Bonnet et al., 2007). As reported previously (Bonnet et al., 2007; Drinnan et al., 1998), differences in detection seem to arise from research centre “traditions/habits”, since considering the four different raters of Basel separately (vs. Liège) did not yield a different picture. Qualitative inspection of the data did not lead to other indications on the origin of the discrepancy between research centres. Despite these discrepancies, Cohen’s κ and inter-rater agreement S are high across HR (and therefore research centres) so that we consider both HR as equally good.

Critically, our results show that AD is performing at least equally as well as HR when comparing against a composite detection of both HR. As it was not possible to objectively select among visually detected arousals those that would be the actual “true” arousals, composite detection was either inclusive (HR inclusive), with all arousals detected by either (or both) HR, or conservative (HR conservative), retaining only those ‘salient’ arousals, common to both HR. The inter-rater agreement measure S (most commonly used though not suitable for unbalanced data) and Cohen’s κ (more conservative but better suited) were always both in the upper ranges indicating high agreement levels. AD detects most of what HR detect, but also picks up many more arousals as reflected by intermediate sensitivity and FDR levels. This is also noticeable for automatic detection of sleep spindles, which are also transient accelerations of EEG intertwined in the rest of the EEG oscillations (Coppieters 't Wallant, Maquet, et al., 2016; Warby et al., 2015). These additional arousals correspond to our mathematic translation of the AASM definition of an arousal (Iber et al., 2007) so they bear the characteristics of an arousal but their visual detection would be difficult, if at all possible. This statement is supported by the fact that arousals that were only detected automatically had lower relative power in the alpha and beta bands, and higher power in the theta band. Increases in the theta band can indeed arguably be harder to disentangle from sleep background activity. This raises the question of what defines an arousal. Can arousals only be detected visually and therefore should one accept all the subjectivity and inter-rater variations? Should one keep on spending a lot of time in detecting arousals in a manner that is not outperforming an automatic tool? We consider that AD has a clear added value as it spares time and increases reproducibility through an algorithmic definition of an arousal. In contrast to visual detection,

it will always yield the same detection when using the same dataset, and its detection bias will be systematic across research centre and study sample. Based on the current definition of an arousal where, for instance, there is no objective threshold determining how large the frequency shift for an event to be classified as an arousal, there is no reason to consider that events that are only detected by AD algorithm do not constitute arousals.

Although AASM recommendations state that arousals during NREM sleep do not need to be associated with changes in EMG tone (M. Bonnet et al., 1992), they might be easier to detect visually if immediately preceded or concomitant to muscular activity change. When we only considered arousals with EMG changes, AD detected much less arousals (from 200 ± 43 to 69 ± 23 ; see Table 3) which leads to FDR levels comparable to inter-HR agreements. This positive effect is counterbalanced by an inevitable reduction in sensitivity, since there are fewer detected events. One is therefore facing 2 options when using our algorithm: either considering all detected arousals, even though a large part would not be visually detected, or considering only those that are associated with EMG changes, and acknowledging that a larger part of what would be visually detected is missing. Importantly, however, we found that AD and HR arousal density over the whole night are correlated, particularly when using HR conservative as gold standard, meaning that an individual with more arousals following visual inspection will also have a lot of AD arousals relative to other individuals of a sample. This tells that AD can be used to characterise arousals in a recording of a dataset and compute correlations with other variables of interest (e.g. subjective sleep quality measures, cognitive assessments, genetic background, etc.). In addition, our dataset included 35 recordings of individuals of both sexes and almost equally split between young (< 30 y) and older (50-70 y) adults. Although we did find differences in some agreement coefficients according to age, particularly when comparing against HR inclusive, we consider that this is likely due to the difference between age groups in the amount of arousals detected by BAS, as arousal density was similar across age-groups when using AD, as well as for Liège HR. The origin of the discrepancy between BAS on the one hand and DC and AD on the other regarding age is unclear but may be related in part to the slight difference in methodology where BAS raters had access to the age of the participants, together with their sex and to the full visual sleep staging (vs. NREM, REM, WAKE for DC). Crucially, sleep stage did not affect detection quality.

Other automatic detection tools of arousals are available. Some are based on ICA (Crespo-Garcia et al., 2008) but retain a subjective component in the selection of the principal

component to attribute to arousals. In addition the ICA tools do not allow to count arousals, but rather to remove their bias in the processing of the data. Tools using temporal and spectral aspect such as our algorithm are also available. Some were only confronted to a single expert rating or ratings from a single research centre (Agarwal, 2005; Cho et al., 2005; De Carli et al., 2004). In addition, previous studies rely on agreement measures which are inappropriate for detection of unbalanced event, i.e. where an event (e.g. arousal event) is much less represented compared to another (non-arousal event). In our view, the use of the more conservative Cohen's κ thus strengthens the message from the present study.

Although constituting an important aspect of the sleep EEG, the current definition of arousals does not allow for a clear consensus on their visual detection as shown by variable detection across HR and research centres. Here, we provide a novel arousal detection tool which comes in addition to these previous tools and reaches agreement levels with HR comparable to HR among themselves. It has the advantage of not requiring a training phase or supervision, it is a fast and objective method of arousal detection. Our dataset only included undisturbed sleep of healthy individuals screened for sleep disorders so that, aside for occasional hypopnoea/apnoea or movement, the vast majority of detected arousals occurred spontaneously. While the literature does not tell whether spontaneous arousals may differ from induced arousals, our algorithm's performance should be assessed in sleep-related pathologies (e.g. apnoea) to further enlarge its validation.

Results

Chapter VI: Heterogeneity in the links between sleep arousals, amyloid-beta and cognition

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This chapter was published as a research article in *JCI Insight* 2021, vol.6(4), e152858..

ABSTRACT

Background. Tight relationships between sleep quality, cognition and amyloid-beta ($A\beta$) accumulation, a hallmark of Alzheimer's disease (AD) neuropathology, emerge in the literature. Sleep arousals become more prevalent with ageing and are considered to reflect poorer sleep quality. Yet, heterogeneity in arousals has been suggested while their associations with $A\beta$ and cognition are not established.

Methods. We recorded undisturbed night-time sleep with EEG in 101 healthy individuals in late midlife (50-70y), devoid of cognitive and sleep disorders. We classified spontaneous arousals according to their association with muscular tone increase (M+/M-) and sleep stage transition (T+/T-). We assessed cortical $A\beta$ burden over earliest affected regions via PET imaging, and cognition via extensive neuropsychological testing.

Results. Arousal types differed in their oscillatory composition in theta and beta EEG bands. Furthermore, T+M- arousals, which interrupt sleep continuity, were positively linked to $A\beta$ burden ($p=.0053$, $R^2\beta^*=0.08$). By contrast, more prevalent T-M+ arousals, upholding sleep continuity, were associated with lower $A\beta$ burden ($p=.0003$, $R^2\beta^*=0.13$), and better cognition, particularly over the attentional domain ($p<.05$, $R^2\beta^*\geq 0.04$).

Conclusion. Contrasting with what is commonly accepted, we provide empirical evidence that arousals are diverse and differently associated with early AD-related neuropathology and cognition. This suggests that sleep arousals, and their coalescence with other brain oscillations during sleep, may actively contribute to the beneficial functions of sleep. This warrants re-evaluation of age-related sleep changes and suggests that spontaneous arousals could constitute a marker of favourable brain and cognitive health trajectories.

INTRODUCTION

Sleep is central to health and cognition, and deteriorates with ageing (C Bassetti, Dogas, & Peigneux, 2014). In addition, sleep disruption is associated with Alzheimer's disease (AD), as assessed by hyper-phosphorylated tau and amyloid-beta (A β) brain accumulation, most likely in a bidirectional manner (Ju, Lucey, & Holtzman, 2014; Van Egroo, Narbutas, Chylinski, Villar González, Maquet, et al., 2019a). Poorer sleep quality (Spira et al., 2013), daytime sleepiness (Carvalho et al., 2018), reduced slow-wave sleep (Ju et al., 2017; Mander et al., 2015), and sleep deprivation (Lucey et al., 2018; Ooms et al., 2014) have been linked to higher A β levels, but also to poorer cognitive performance (Mander et al., 2015; Scullin & Bliwise, 2015).

Sleep arousals, defined as transient accelerations in sleep electroencephalogram (EEG) rhythms, are usually considered as brain reactions to internal (e.g., apnoea) or external (e.g., auditory stimulus) perturbations (Halasz et al., 2004). Although key elements of sleep microstructure, they can also shape its macrostructure and lead to a shallower sleep stage (Iber et al., 2007). They are most often considered as markers of sleep disruption, thereby a detrimental and harmful sleep feature. Several conceptual definitions classified them almost exclusively in the context of sleep disorders (e.g. sleep disordered breathing – SDB, and to a smaller extent periodic limb movements syndrome – PLMS), or in experimental protocols inducing arousals through external – mainly using auditory – stimulation (Philip et al., 1994; Rudzik et al., 2018). These types of studies yielded mixed results. A negative link between arousal prevalence during sleep and cognitive performance was revealed in SDB, particularly in attention, and sometimes the executive and memory domains (Aloia et al., 2004). By contrast, other investigations did not find such a relationship, and imputed alterations in cognition in SDB to brain hypoxia (see (Aloia et al., 2004) for review). In individuals devoid of sleep pathologies, arousals evoked by auditory stimuli were reported to impact subsequent daytime alertness (Roehrs et al., 1994). In addition, sleep fragmentation induced by auditory stimulation is associated with higher A β cerebrospinal fluid (CSF) content the following day (Ju et al., 2017).

Importantly, spontaneous arousals, i.e. not elicited by any identifiable internal or external stimuli, also constitute an authentic element of undisturbed sleep in healthy individuals. Their mechanisms, cerebral correlates and functional consequences remain largely unknown (Halasz et al., 2004) with some authors suggest that there may be physiologic and

pathologic arousals. Understanding their respective roles might shed light on the adaptive properties of the sleeping brain and provide insight into pathological mechanisms associated with sleep disturbances (Halasz et al., 2004).

Here, we assessed whether different types of spontaneous arousals during sleep were differentially associated with A β cortical deposition and cognitive performance in a cohort of healthy individuals in late midlife. We were able to tease apart different types of arousals, based on their temporal relationships with increased muscular tone and sleep stage transitions. In line with the hypothesis that arousals perturb sleep, we anticipated that arousals fragmenting sleep structure would be associated with both worse cognitive performance and A β deposition in brain areas that are first affected by this AD-related neuropathological process.

RESULTS

EEG oscillations differ across arousal types

We recorded undisturbed sleep at habitual sleep times under electroencephalogram (EEG) in 101 healthy individuals aged 50 to 70 y (59 ± 5 y; 68 women), following one week of regular sleep-wake schedule (Figure 1). In order to evaluate the potential heterogeneity of arousals, we split them according to two criteria, which we considered as relevant in research settings as well as clinical practice. We first chose to focus on whether arousals did trigger a sleep stage transition (T+) (when they occurred within 15s of a stage change) or not (T), as arousals may or may not lead to a lighter sleep stage (Iber et al., 2007) and they have already been investigated in that regard (Stepanski, Lamphere, Badia, Zorick, & Roth, 1984). Secondly, we considered their salience, reflected by the concomitant increase in electromyogram (EMG) tone (M+) or its absence (M-), as it is among the arousal scoring criteria (needed to score an arousal in REM sleep).

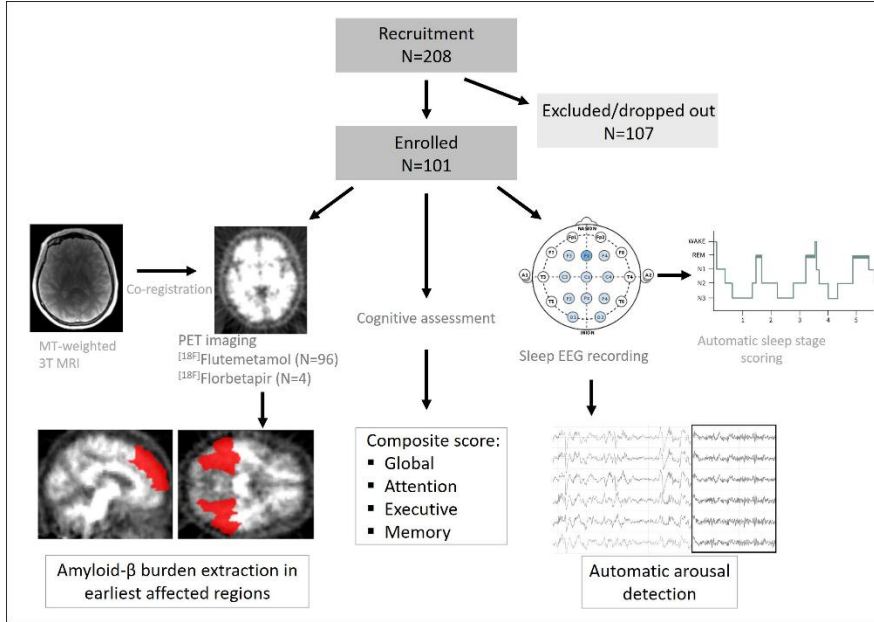


Figure 1. Overview of the study. 208 participants were recruited, of which 107 did not participate in the study, as they were excluded based on inclusion criteria (see methods section), if sleep apnoea were detected (>15/h) or decided to withdraw. Participants underwent $[^{18}\text{F}]$ Flutemetamol (N=96) / $[^{18}\text{F}]$ Florbetapir (N=4) PET scan to assess A β burden, which we extracted over the earliest affected regions; they were also tested via an extensive battery of neuropsychological tasks from which we extracted global score, as well as performance over 3 main cognitive domains (attention, executive and memory); and habitual sleep was recorded via EEG from which arousals were automatically detected.

In a first step, we assessed whether characterising sleep arousals by their association with sleep stage transition (T+ or T-), and the co-occurrence of an EMG tone increase (M+) or not (M-) resulted in differences in their oscillatory properties. We computed individual relative power in the different EEG frequency bands defining an arousal (theta - 4.5-7.5Hz, alpha - 8.5-11.5Hz, and beta - 16.5-29.5Hz). A generalised linear mixed model (GLMM) with relative power as dependent variable (and adjusted for data distribution), first indicated that relative power changed across frequency bands ($F_{2,294.1}=403.84$, $p<0.0001$, $R^2_{\beta^*}=0.73$). More importantly, it yielded a triple interaction between transition, EMG status and frequency band ($F_{2,880}=3.39$, $p=0.034$, $R^2_{\beta^*}=0.008$) implying that arousals differ in their spectral composition based on the presence or absence of EMG changes and sleep stage transition (Figure 2A). This was further reflected in main effects of EMG status ($F_{1,989.9}=75.97$, $p=0.0001$, $R^2_{\beta^*}=0.07$) and transition ($F_{1,708.6}=39.17$, $p<0.0001$, $R^2_{\beta^*}=0.05$), as well as in interactions between transition

and frequency band ($F_{2,709.5}=34.62$, $p<0.0001$, $R^2_{\beta^*}=0.09$), between EMG status and frequency band ($F_{2,979.2}=187.39$, $p<0.0001$, $R^2_{\beta^*}=0.28$), and between EMG status and transition ($F_{1,879.3}=22.55$, $p<0.0001$, $R^2_{\beta^*}=0.025$). Based on this first analysis, we therefore concluded that the factors of arousal heterogeneity eloquently define 4 types of arousals (T+M+, T+M-, T-M+, T-M-). We finally note that multiple post-hoc comparisons within each band yielded significant differences across arousal types over the theta and beta bands (see Table 1).

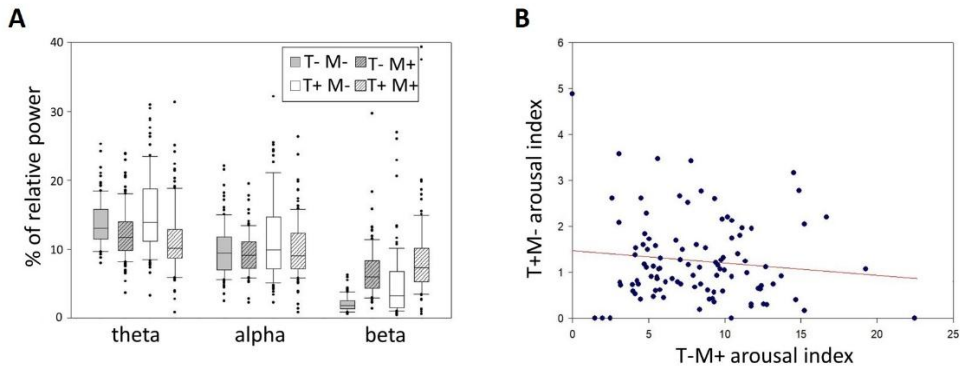


Figure 2. (A) Spectral composition of arousal types. Box plot of relative power in the theta (4.5-7.5Hz), alpha (8.5-11.5Hz) and beta (16.5-29.5Hz) band for T-M-, T-M+, T+M- and T+M+ arousals with error bars. The boxes' central line indicates the median of power values, with the bottom and upper edges showing the 25th and 75th percentiles, respectively. T: arousal associated (T+) or not (T-) with sleep stage transition; M: arousal associated (M+) or not (M-) with an increase in EMG tone. Indexes correspond to hourly prevalence. **(B) Absence of significant correlation between T-M+ arousals and T+M- arousals (Spearman $r=-0.05$ $p=.60$).**

Table 1. Post-hoc comparisons of the relative power for each arousal type within each frequency band.

Arousal type		vs. arousal type		Frequency band relative power					
				Theta		Alpha		Beta	
M	T	M	T	t value	Adj p	t value	Adj p	t value	Adj p
-	-	-	+	-1.03	0.73	-2.02	0.18	-10.26	<.0001
-	-	+	-	1.99	0.19	0.01	1.00	-18.92	<.0001
-	-	+	+	4.08	0.0003	-0.35	0.99	-20.91	<.0001
-	+	+	-	3.30	0.006	2.02	0.18	-11.41	<.0001
-	+	+	+	5.11	<.0001	1.39	0.51	-12.73	<.0001
+	-	+	+	2.61	0.005	-0.43	0.97	-3.29	0.006

T: arousal associated (T+) or not (T-) with sleep stage transition; M: arousal associated (M+) or not (M-) with an increase in EMG tone. Indexes correspond to hourly prevalence. Significant contrasts are in bold

Arousals heterogeneity reflects different associations with A β burden

In line with our goal to consider very early AD-related neuropathological process, A β burden was quantified over the regions previously reported as the earliest cortical aggregation sites (Grothe et al., 2017), that is the frontal medial cortex and basal part of temporal lobe (fusiform & inferior temporal gyri) in all but one participant. We tested in a GLMM whether associations between arousal density varied with transition (T+, T-) and EMG (M+, M-) statuses and were associated with A β burden, while regressing out age and sex effects. We first observed a main effect of transition ($F_{1,196}=607.52$, $p<0.0001$, $R^2_{\beta*}=0.76$) and EMG status ($F_{1,98}=70.63$, $p<0.0001$, $R^2_{\beta*}=0.42$) as well as an interaction between EMG status and transition ($F_{1,196}=102.32$, $p<0.0001$, $R^2_{\beta*}=0.34$), indicating that density of arousal types significantly varied. Interestingly, we did not find any significant main effect of early cortical A β burden ($F_{1,96}=0.26$, $p=0.61$), age ($F_{1,96}=2.44$, $p=0.12$) and sex ($F_{1,96}=0.12$, $p=0.73$). Critically, the GLMM yielded a significant triple interaction between early cortical A β burden, EMG status and transition ($F_{1,196}=7.16$, $p=0.008$, $R^2_{\beta*}=0.035$) implying that the association between arousals and early cortical A β burden depends on the concomitant change in muscular tone and sleep stage transition. The heterogeneity in spontaneous arousals was further reflected by the significant interactions between early cortical A β burden and EMG status ($F_{1,98}=8.64$, $p=0.004$, $R^2_{\beta*}=0.08$). Figure 3 decomposes the associations between each of the four types of arousals and early cortical A β burden.

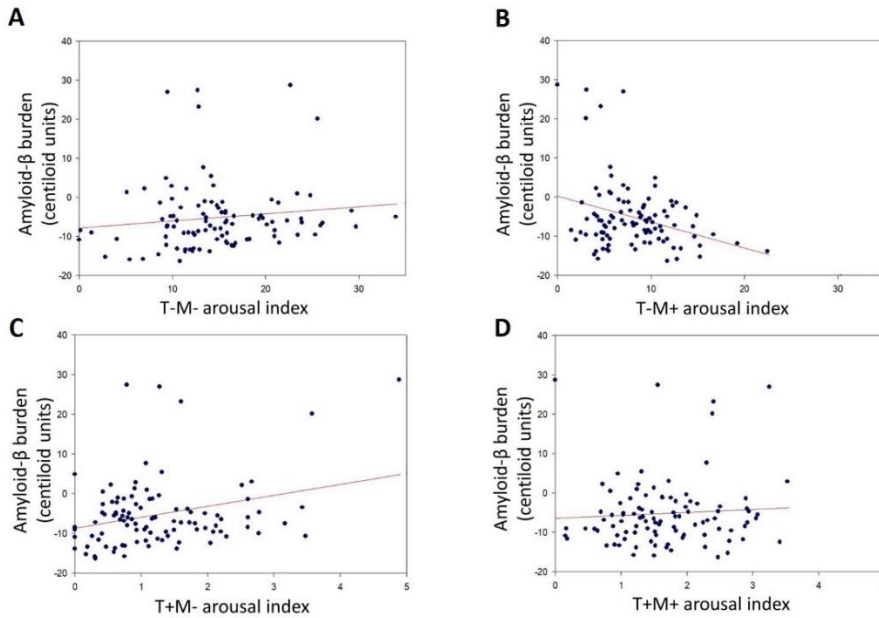


Figure 3. Associations between prevalence of different types of arousals and early cortical A β burden. (A) no correlation between T-M- arousals and A β burden (Spearman $r=.16$, $p=.11$); (B) significant negative correlation between T-M+ arousals and A β burden (Spearman $r=-.16$, $p=.11$); (C) significant positive correlation between T+M- arousals and A β burden (Spearman $r=.17$, $p=.08$); (D) no correlation between T+M+ arousals (Spearman $r=.07$, $p=.46$). See main text for full GLMM output controlling for several covariates. T: arousal associated (T+) or not (T-) with sleep stage transition; M: arousal associated (M+) or not (M-) with an increase in EMG tone. Indexes correspond to hourly prevalence.

We further computed a GLMM with early cortical A β burden as dependent variable to explore whether its association with T-M+ and T+M- arousal truly differed in the part of A β burden variance they explained in a more complex model, regressing out age and sex. Both associations were significant with a negative link between A β and T-M+ arousals ($F_{1,95}=14.15$, $p=0.0003$, $R^2_{\beta*}=0.13$) and a positive association between A β and T+M- arousals ($F_{1,95}=8.16$, $p=0.0053$, $R^2_{\beta*}=0.08$) – together with an expected main effect of age ($F_{1,95}=13.02$, $p=0.0005$, $R^2_{\beta*}=0.12$) (Jack et al., 2017), and no main effect of sex ($F_{1,95}=2.54$, $p=0.11$). Critically, a post-hoc contrast showed that the links between the two types of arousals and early cortical A β burden were significantly different ($t_{93}=3.73$, $p=0.0003$). In addition, T-M+ and T+M- arousals were not correlated (Figure 2B). Supplementary analysis showed the same statistical picture in a GLMM including all four arousal types together, with a significant post-hoc contrast when

considering T-M+ and T+M- arousals vs. early cortical A β burden (Table 2). Results were not driven by arousals occurring in non-Rapid Eye Movement (NREM) or REM sleep as statistical outputs were the same if we considered the four types of arousals in NREM/REM separately (M+ only in REM as arousal definition is REM requires change in muscle tone) (Table 2).

Table 2. Output of the GLMM with A β burden as dependent variable, and arousal types.

When considering arousals in all sleep stages together						
	Age	Sex	T-M-	T-M+	T+M-	T+M+
A β burden	F = 11.98	F = 2.11	F = 0.00	F = 15.22	F = 3.22	F = 2.02
	p = 0.0008	p = 0.15	p = 0.99	p = 0.0002	p = 0.076	p = 0.16
	R²β*=0.11			R²β*=0.14		
T-M+ T+M- contrast: t = -2.71, p = 0.008, adjusted = 0.048, estimate = -2.87						

When considering arousals in NREM/REM separately						
	Age	Sex	T-M- NREM	T-M+ NREM	T+M- NREM	T+M+ NREM
A β burden	F = 12.57	F = 2.59	F = 0.13	F = 11.94	F = 3.54	F = 1.05
	p = 0.0006	p = 0.11	p = 0.72	p = 0.0008	p = 0.06	p = 0.31
	Age	Sex	T-M+ REM		T+M+ REM	
A β burden	F = 8.55	F = 0.63	F = 5.95		F = 0.14	
	p = 0.0043	p = 0.43	p = 0.017		p = 0.71	

All F tests had 1 (main effect) and 93 (error) degrees of freedom; except for the models with arousals in NREM/REM, which had 1 (main effect) and 95 (error) degrees of freedom. Significant associations are in bold and are accompanied by their corresponding Semi-partial R² (R² β *).

Arousals linked with better A β status are associated with better cognitive performance

We then tested whether cognition, as assessed in a global index through an extensive neuropsychological test battery, was differentially associated with the two arousal types showing opposite association with early cortical A β burden. In a GLMM, the association between global cognition and T-M+ arousal index was found to be significant (p=0.048, R² β *=0.04), on top of the education effect, but no relation with T+M- arousal index (p=0.25), age, or sex (Table 3; Figure 4) was found. Additional exploratory GLMMs with each specific cognitive domain in turn showed this association was driven by the attentional domain

($p=0.032$, $R^2_{\beta^*}=0.047$), and was not significant for the executive ($p=0.09$) or memory domains ($p=0.91$).

Table 3. Outputs of GLMMs assessing associations between cognitive performances (global and specific domains-dependent variables) and arousal types, while adjusting for age, sex and education (independent variables).

	T-M+ arousal index	T+M- arousal index	Age	Sex	Education
GLOBAL	F = 4.01 p = 0.048 $R^2_{\beta^*} = 0.04$	F = 1.36 p = 0.25	F = 3.41 p = 0.068	F = 0.11 p = 0.74	F = 10.92 p = 0.0013 $R^2_{\beta^*} = 0.10$
ATTENTION	F = 4.74 p = 0.032 $R^2_{\beta^*} = 0.047$	F = 0.83 p = 0.36	F = 6.08 p = 0.015 $R^2_{\beta^*} = 0.06$	F = 0.10 p = 0.75	F = 4.48 p = 0.037 $R^2_{\beta^*} = 0.045$
EXECUTIVE	F = 2.92 p = 0.09	F = 0.99 p = 0.32	F = 0.92 p = 0.34	F = 0.34 p = 0.56	F = 10.84 p = 0.0014 $R^2_{\beta^*} = 0.10$
MEMORY	F = 0.01 p = 0.91	F = 0.33 p = 0.57	F = 0.08 p = 0.77	F = 3.15 p = 0.08	F = 2.35 p = 0.13

All F tests had 1 (main effect) and 95 (error) degrees of freedom. Significant associations are in bold and are accompanied by their corresponding Semi-partial R^2 ($R^2_{\beta^*}$). T: arousal associated (T+) or not (T-) with sleep stage transition; M: arousal associated (M+) or not (M-) with an increase in EMG tone.

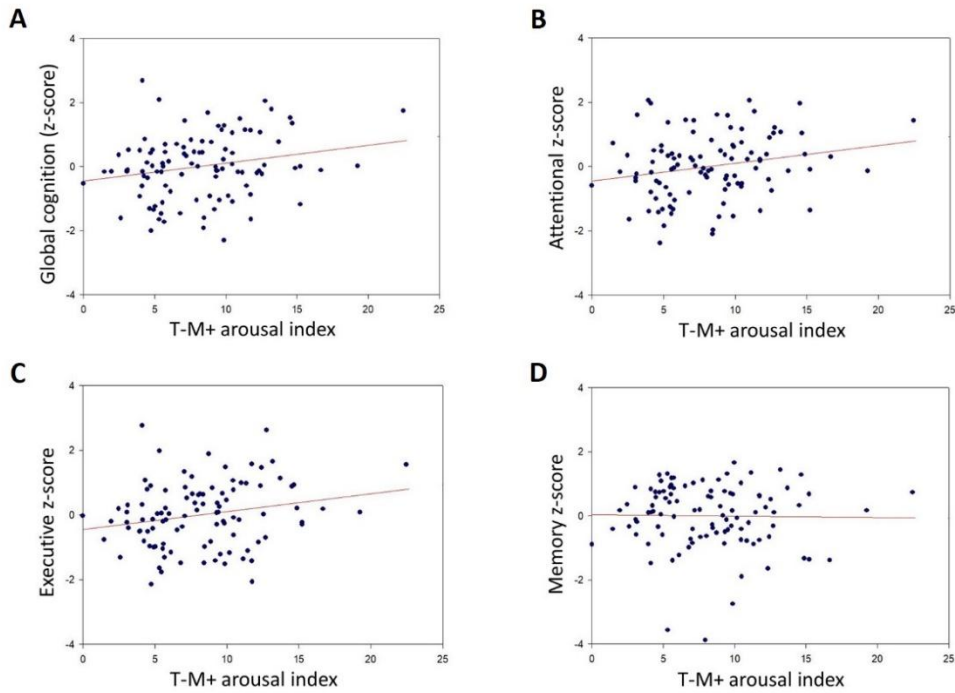


Figure 4. Association between T-M+ arousals prevalence and cognitive performance. (A) T-M+ arousals and global cognition (Spearman $r=.21$, $p=.04$); (B) T-M+ arousals and attention (Spearman $r=.22$, $p=.03$); (C) T-M+ arousals and executive functioning (Spearman $r=.21$, $p=.03$); (D) T-M+ arousals and memory (Spearman $r=-.03$, $p=.71$). See Table 3 for full GLMM outputs. T: arousal associated (T+) or not (T-) with sleep stage transition; M: arousal associated (M+) or not (M-) with an increase in EMG tone. Indexes correspond to hourly prevalence.

We assessed the specificity of the findings for T-M+ arousals and considered the potential link between number of full awakenings during sleep and wake after sleep onset (WASO) and the different cognitive measures in separate exploratory GLMMs. We found no link between cognition and number of awakenings (Figure 5, A-D), while a significant negative association was detected between WASO and global cognition ($F(1,95)=4.66$, $p=0.03$) which was driven by the executive domain ($F(1,95)=7.58$, $p=.007$) (Figure 5, E-H). Furthermore, neither WASO nor number of awakening were associated with early cortical A β burden (Figure 5, I and J).

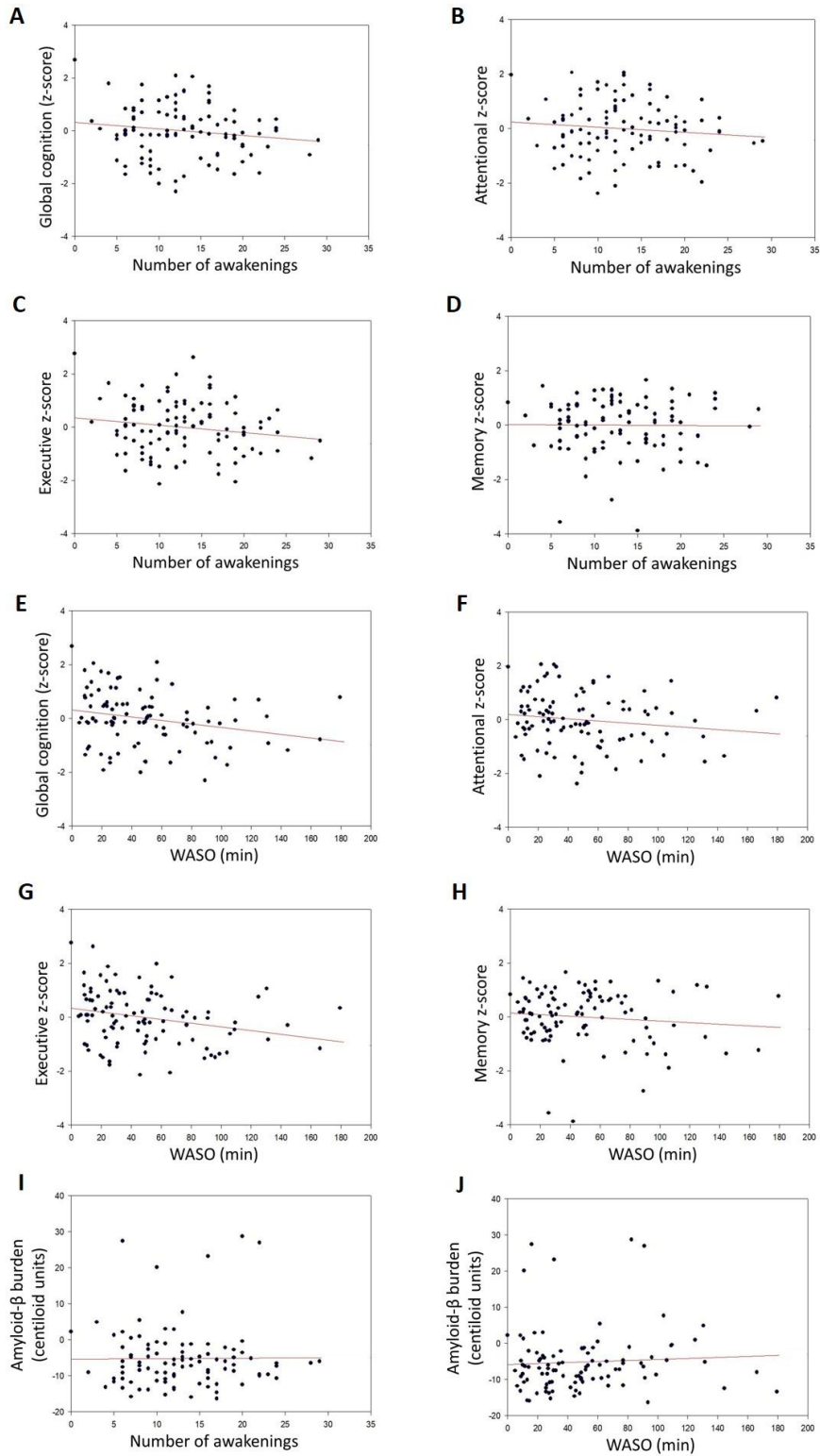


Figure 5. Association between number of awakenings and cognition (A) Global cognitive performance (Spearman $r=-0.10$, $p=0.30$; GLMM $F_{1,95}=2.21$, $p=0.14$); (B) attentional (Spearman $r=-0.07$, $p=0.48$; GLMM $F_{1,95}=0.89$, $p=0.35$); (C) executive (Spearman $r=-0.11$, $p=0.25$; GLMM $F_{1,95}=3.53$, $p=0.06$); (D) memory performances (Spearman $r=-0.03$, $p=0.80$; GLMM $F_{1,95}=0.00$, $p=0.98$); between WASO and cognition (E) Global cognitive performance (Spearman: $r=-0.24$, $p=0.01$; GLMM $F_{1,95}=4.66$, $p=0.03$); (F) attentional (Spearman: $r=-0.17$, $p=0.11$; GLMM: $F_{1,95}=0.56$, $p=0.46$); (G) executive (Spearman: $r=-0.28$, $p=0.005$; GLMM: $F_{1,95}=7.58$, $p=0.007$); (H) memory performances (Spearman: $r=-0.06$, $p=0.59$; GLMM: $F_{1,95}=1.11$, $p=0.30$); between early cortical A β burden and (I) number of awakenings (Spearman: $r=0.01$, $p=0.90$; GLMM $F_{1,95}=0.22$, $p=0.64$); (J) WASO (Spearman: $r=0.11$, $p=0.28$; GLMM $F_{1,95}=0.05$, $p=0.83$).

DISCUSSION

Brain dynamics which buttress cerebral functions entail stationary and non-stationary interactions between neuronal populations (Friston, 2000a). Sleep stages, which can be seen as enduring and widespread oscillatory modes sculpting brain activity, allow recurrent brief faster oscillatory activity, which sometimes lead to stage transitions (Dang-Vu et al., 2008; Tononi & Cirelli, 2014). Here, we focused on spontaneous arousals because their functional correlates remain undetermined. They are usually considered to induce sleep disruption and its detrimental functional consequences. However, spontaneous sleep arousals, i.e. not elicited by identifiable event, might also carry positive effects on brain functions. We quantified the prevalence of spontaneous arousals during undisturbed sleep in healthy individuals in late midlife, and assessed whether it was associated with early cortical A β deposition and cognitive performance. Based on the theoretical concept that sleep arousals are diverse (Halasz et al., 2004), we classified them according to their temporal association with a change in muscular tone and a sleep stage transition. These criteria were deemed clinically relevant as arousals may or may not affect sleep macrostructure while muscular tone constitutes an arousal marker in REM. Based on this straightforward phenotyping in a large data sample we provide the first empirical evidence that different types of sleep arousals have distinct correlates in terms of cognition and brain amyloid burden. Indeed, we found that arousals associated with sleep transitions (T+M-) are associated with higher cortical A β deposition in brain regions affected early on by AD neuropathology, suggesting their association with sleep fragmentation and worse brain status. By contrast, the more prevalent T-M+ arousals, which do not result in sleep transitions, are all the more frequent as A β deposition is low and cognitive performance superior, particularly in the attentional domain. This arousal type is therefore associated to a

more favourable brain and cognitive status. Although sizes of the effects we detected remain modest, enduring small phenomenon can shape lifelong trajectories. The present findings may therefore be of particular importance since arousals have been reported to increase with age, and age represents the most important risk factor for cognitive decline and AD (Van Egroo, Narbutas, Chylinski, Villar González, Maquet, et al., 2019a).

Our analyses show that the main characteristic differentiating the two types of arousals is whether or not they lead to a sleep stage transition. A second important criterion consisted of the concomitant increase in EMG tone. Aside from their different links with A β burden and cognition, T+M- and T-M+ arousals are not correlated with each other and differ in their spectral composition: T+M- bear a larger proportion of theta power while T-M+ arousals are composed of a higher proportion of beta power. The reason why T-M- and T+M+ arousals are not significantly associated with A β and cognition is unclear and might reside in different prevalence or in diverging effects of sleep transitions and EMG bursts, which would hinder the relationship. Future studies are warranted to further investigate this issue.

Two hypotheses can be put forward to explain the heterogeneity in arousals. On the one hand, all arousals, triggered by a common set of brain areas, might be part of a continuum in which each arousal is characterised by the intensity in its driving neural activity, its spectral composition, its associated muscular tone and its probability of sleep stage transition. Alternatively, the two arousal types are distinct physiological events prompted by different triggering brain structures and propagation cerebral networks. Oddly enough, the origin of spontaneous arousals remains elusive. Recent fMRI data showed that subcortical regions (including the thalamus, midbrain, basal ganglia and cerebellum) were activated during non-REM arousals while cortical regions were deactivated (Zou et al., 2019). A recent yet-to-be-reviewed study in rodents provides evidence that arousals leading to sleep state transition are, at least partly driven by the locus coeruleus (LC), brainstem source of norepinephrine with strong and ubiquitous influence on distant cortical brain regions, including during sleep (Kjaerby et al., 2020). In addition, optogenetic stimulation of the LC causes immediate sleep-to-wake transitions, from both NREM and REM sleep and results in high-frequency EEG activity (Carter et al., 2010). Hence, subcortical activity, for instance in the LC, could underlie transition-arousals while no-transition arousals could also merely be the reflection of cortico-cortical or thalamo-cortical interplay (Halasz et al., 2004). Identifying the brain sources of the two types of arousals would require invasive animal testing, coupling EEG to fMRI recordings in humans, or source reconstruction of high density EEG signals (Dang-Vu et al., 2008).

The cellular and molecular underpinnings of the distinct relationship between the two types of arousals, A β burden, and cognition are currently unknown. We can reasonably speculate that T+M- arousals have 2 potentially deleterious impacts. Firstly, they interrupt a sleep stage and consequently all its associated cellular phenomena, like plasticity (Tononi & Cirelli, 2014). Secondly, it seems possible that they considerably increase cellular activity in diffused cerebral regions, a condition conducive to increase A β release. By contrast, one could tentatively speculate that T-M+ arousals promote A β clearance, hypothetically by increasing the pulsatility of cortical penetrating arteries (Plog & Nedergaard, 2018). Additionally, T-M+ arousals might offer recurring opportunities to transiently synchronise distant brain areas, in frequency bands otherwise related to cognition during wakefulness (e.g. beta oscillations (Spitzer & Haegens, 2017)) without enduringly disrupting the underlying brain oscillations (i.e. sleep state), similarly to what sleep spindles allow over sigma band (12-16Hz) oscillations (Mircea Steriade, 2003). In complex dynamics wordings, T-M+ arousals can be seen as distinct dynamics generated when the oscillatory trajectory is trapped in a local submanifold of an attractor (Friston, 2000b), meaning the arousal would represent only a temporary breakout from the global oscillatory regime. These transient oscillations give rise to dynamic instability despite the fact that the global manifold does not change. Dynamic instability is a form of complexity in neuronal systems, which is critical for adaptive brain functions such as selection in self-organising systems, learning or memory (Friston, 2000a). On the other hand, T+M- arousals would represent a distinct type of complexity, where the involvement of the brainstem would lead to a change in oscillatory regime through a change in the attractor manifold. Similar transient oscillations have been previously reported during wakefulness and related to cognition (Friston, 2000a). Further studies are needed to unravel whether higher T+M-/lower T-M+ arousal indexes are facilitating A β aggregation or if, conversely, accumulating A β burden is disrupting sleep processes (Van Egroo, Narbutas, Chylinski, Villar González, Maquet, et al., 2019a). Data in young individuals, in which current A β detection is typically negative (Grothe et al., 2017), as well as longitudinal studies are needed to address this issue.

We emphasise that (1) our cohort only comprised healthy individuals, devoid of SDB, and (2) we focused on spontaneous arousals, which are not generated in response to detectable endogenous or exogenous perturbation (e.g. apnoea or noise). Therefore, our findings probably do not apply to potentially more prevalent perturbation-induced arousals and their negative behavioural (Aloia et al., 2004; Roehrs et al., 1994) and neurodegenerative aftermaths (Ju et al., 2017). We also underline that we aimed to investigate links between sleep and A β burden

early on in this neuropathological process, therefore our volunteers did not show large A β deposition (only 5 could be considered as A β positive). Although A β is a hallmark of AD neuropathology, we do not know which volunteer will develop AD and therefore which one can be considered to undergo a true AD process or an AD-like or AD-related process. As for any A β signal, its predictive value remains debated. It is tantalising to suggest, and empirically testable, that arousals found in SDB mostly consist in transition-arousals which would contribute in part to the higher risk for AD reported in SDB (Bubu et al., 2018). We further found no significant link between early A β burden and the number of full night-time awakenings during sleep or with time spent awake after sleep onset, two markers related to the fragmentation of sleep macrostructure defining in part sleep quality. The associations we find with A β burden in healthy late midlife appear therefore to be stronger with, if not specific to, sleep arousals, as compared to other indices of wakefulness during sleep or fragmentation of sleep. This contrast with a previous actigraphy study that reported correlations between WASO and A β burden in participants older than those included here (mean: 76.7 $\pm$ 3.5y) (Ettore et al., 2019). Our findings may therefore suggest that, at a younger age (~59y), the detrimental association between sleep quality and AD neuropathology initially concerns transition-arousals leading to sleep macrostructure fragmentation, before being subsequently detected over other markers of sleep fragmentation.

Sleep arousals may connect the sleeper's brain with the surrounding endogenous and exogenous relevant incoming information and contribute to elements of cortico-cortical information processing (Friston, 2000b; Halasz et al., 2004) as done through sleep spindles, another fundamental feature of sleep microstructure (Steriade, 2003). In other words, our findings suggest that sleep arousals, and their coalescence with other brain oscillations during sleep, may actively contribute to the beneficial functions of sleep. Arousals may interact with spindles and slow waves, however, so that we cannot rule out a contribution of these events to the effects we report. Future research should assess whether arousals are predictors of A β burden independent of other known neurophysiological elements/oscillations of sleep linked to cognition and brain health. Visual inspection of the data indicates that despite occasional co-occurrence, slow wave are not strongly nor systematically associated with any type of arousals. Our findings constitute the first empirical evidence of the conceptual existence of different arousal types differently associated to important parameters of cognitive and brain health (Halasz et al., 2004). Sleep micro-fragmentation, as easily indexed by automatic detection of spontaneous arousals, could therefore potentially constitute a marker of favourable

brain and cognitive trajectory in clinical practice, at least in late midlife adults and/or in individuals with still early AD-related neuropathology.

SUBJECTS/MATERIALS AND METHODS

Study design and participants

In order to target early A β brain deposit (Grothe et al., 2017), we recruited healthy older individuals aged 50-70y. 208 volunteers were recruited, of which 101 participated in the actual study (Table 4), the rest being excluded due to one of the following exclusion criteria: clinical symptoms of cognitive impairment (Dementia rating scale <130; Mini Mental State Examination <27); Body Mass Index (BMI) ≤ 18 and ≥ 29 ; recent psychiatric history or severe brain trauma; documented/diagnosed sleep pathologies such as insomnia and REM behaviour disorder; 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 last 2 months.

Table 4. Sample characteristics of our dataset (mean $\pm$ SD) N=101.

Sex	68 ♀ / 33 ♂
Age (y)	59.4 $\pm$ 5.3
Education (y)	15.2 $\pm$ 3
Race	White
Dementia rating scale (N=97)(Mattis, 1976)	142.5 $\pm$ 1.9
BMI (kg/m ²)	24.6 $\pm$ 2.9
Apnoea/hypopnoea index (nb/hr)	3.1 $\pm$ 2.9
PLMs (nb/hr)	5.3 $\pm$ 15.4
TST (min)	393.2 $\pm$ 45.9
WASO (min)	49.3 $\pm$ 37.2
Awakenings index (nb/hr)	1.7 $\pm$ 0.8
% N1	6.2 $\pm$ 2.7
% N2	51.6 $\pm$ 8.8
% N3	19.1 $\pm$ 6.4
% REM	23.1 $\pm$ 6.8
Total arousal index (nb/hr) $\pm$ SD [range]	27.6 $\pm$ 9
* T- arousal index (nb/hr) $\pm$ SD [range]	23 $\pm$ 8 [2-46]

* T-M- index (nb/hr) $\pm$ SD [range]	15 $\pm$ 6 [0-34]
* T-M+ index (nb/hr) $\pm$ SD [range]	8 $\pm$ 4 [0-22]
* T+ arousal index (nb/hr) $\pm$ SD [range]	3 $\pm$ 1 [0-6]
* T+M- index (nb/hr) $\pm$ SD [range]	1 $\pm$ 1 [0-5]
* T+M+ index (nb/hr) $\pm$ SD [range]	2 $\pm$ 1 [0-4]
* M- arousal index (nb/hr) $\pm$ SD [range]	16 $\pm$ 7 [0-36]
* M+ arousal index (nb/hr) $\pm$ SD [range]	10 $\pm$ 4 [0-24]

T: arousals associated (T+) or not (T-) with a sleep stage transition; M: arousals associated (M+) or not (M-) with an increase in EMG signal.

Participants were screened for sleep apnoea/hypopnoea syndrome during an in-lab night of sleep under polysomnography (PSG) preceding the one that was analysed in the results section of this paper. This PSG included EEG (Fz, Cz, C3, PZ, Oz), 2 bipolar electrooculogram (EOGs), and 2 bipolar submental EMG electrodes, 2 bipolar electrocardiogram (ECGs), 2 sets of bipolar leg electrodes, thorax and abdominal belts, an oximeter, a nasal canula as well as a snoring sensor. As is typically done in similar sleep studies (André et al., 2020; Mander et al., 2015), volunteers with an apnoea/hypopnea index ≥ 15 /h were excluded (79 subjects had an AHI ≥ 0 and <5 ; 19 subjects had an AHI ≥ 5 and <10 ; 3 subjects had an AHI ≥ 10 and <15). Given the low arousal index of our volunteers and the low rate of PLMS in our sample (9 subjects had a PLM index ≥ 15), and given that controlling for those two covariates did not change the statistically significant associations we found in the reported models, we did not include them in the statistical analyses reported below. One volunteer was excluded from analyses that included amyloid-beta data due to corrupted PET-scan data caused by technical issues during acquisition. Demographic characteristics of the study sample can be found in Table 4.

Sleep assessment

Participants came to the lab for an adaptation night under polysomnography after which those with sleep an apnoea/hypopnoea index ≥ 15 /h were excluded from further participation. Volunteers were required to follow a regular sleep-wake schedule (± 30 min) for 1 week based on their preferred bed and wake-up times before sleep EEG recording, in order to record their sleep in settings as close as possible to habitual conditions. Compliance was verified using sleep diaries and wrist actigraphy (Actiwatch©, Cambridge Neurotechnology,

UK). Participants then joined the laboratory ~6.5h prior to habitual sleep time and were maintained in dim-light thereafter. Undisturbed habitual sleep was recorded with N7000 amplifiers (EMBLA, Natus, Planegg, Germany) using 11 EEG derivations placed according to the 10-20 system (F3, Fz, F4; C3, Cz, C4; P3, Pz, P4; O1, O2), 2 bipolar electrooculogram (EOGs), and 2 bipolar submental electromyogram (EMG) electrodes. Recordings were sampled at 200 Hz, and re-referenced to the mean of the two mastoids.

Arousal detection

Sleep stage scoring and arousal detection were carried out in separate steps by two independent algorithms. Sleep stage scoring was performed in 30s windows using a validated algorithm (ASEEGA, Physip, Paris, France) (Berthomier et al., 2007; L Peter-Derex et al., 2020). Automatic arousal detection was then computed as it is objective, reproducible and time-saving (Chylinski et al., 2020). We used an individually tailored validated algorithm based on the American Academy of Sleep Medicine (AASM) definition (Iber et al., 2007) of arousal but without using sleep stage information. Automatic scorings were visually inspected following computation.

In brief, arousal detection is performed over all electrodes on whole-night recordings split into 1s epoch in two successive steps computed over the power in the broad-alpha (7-13Hz), beta (16-30Hz) and lower-theta (3-7Hz) frequency bands, excluding the sigma band (11-16Hz), i.e. corresponding to frequency of sleep spindles, which cannot be considered as arousals. A fixed threshold is first applied to detect abnormal EEG activity relatively to the whole-night recording: any 1s epoch with power in any of the three frequency bands higher than the whole-night median value in each frequency band is considered as a potential arousal. The second step adapts the threshold to account for the specific EEG background activity in a shorter time window. A specific threshold is computed for each 30s window: all 1s epochs without concomitant EMG tone increase are selected, as well as the first ten 1s epochs without EMG increase before and after the 30s window being evaluated; threshold of each frequency band consists in the median power over the selected 1s epochs. Events composed of at least 3 consecutive 1s epochs with changes in EEG frequencies higher than twice the local median and one median of the whole recording for that frequency band were considered as arousals. For detailed explanations on the method, see (Chylinski et al., 2020).

In order to evaluate the potential heterogeneity of arousals, we split them according to two criteria, which we considered as relevant in research settings as well as clinical practice.

Firstly, whether arousals did trigger a sleep stage transition (T+) (when they occurred within 15s of a stage change – in the second half of an epoch preceding a stage change or in the first half of an epoch assigned a different stage than the previous epoch) or not (T-); and secondly, their salience, reflected by the concomitant increase in EMG tone (M+) or its absence (M-).

Spectral analysis of arousals' power was carried out through a time-frequency analysis on the first 3 seconds of arousals using Morlet's wavelet transform in SPM12 (https://github.com/spm/spm12/blob/master/spm_eeg_specest_morlet.m) on Fz electrode. Detrending was done over the 500ms prior to arousal event. Data was then averaged per arousal type prior to summing in the typical EEG bands that may compose an arousal (theta - 4.5-7.5Hz, alpha – 8.5-11.5Hz, and beta – 16.5-29.5Hz). Given the variety of factors that impact total power (e.g. conductivity of the involved tissues, i.e. scalp, skull, CSF etc) (Laarne et al., 1999) and thus renders it complex to compare across subjects, relative power of each band was computed through a normalisation relative to 0.5-to-30Hz total power.

MRI data

MRI data was used in order to determine the region of interest used for extraction of A β burden value based on PET images. Quantitative multi-parametric MRI acquisition was performed on a 3-Tesla MR scanner (Siemens MAGNETOM Prisma, Siemens Healthineers, Germany) to get a magnetization transfer (MT)-weighted contrast, based on multi-echo 3D fast low angle shot at 1 mm isotropic resolution (Weiskopf & Helms, 2008) (with flip angle = 6° and application of additional off-resonance Gaussian-shaped RF pulse). MRI multi-parameter maps were processed with the hMRI toolbox (Tabelow et al., 2019) (<http://hmri.info>) and SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK) to obtain a quantitative MT map and segmented images (grey matter, white matter, CSF), normalised to the standard MNI space using unified segmentation (Ashburner & Friston, 2005).

PET-scan

A β PET imaging was performed using [^{18}F]Flutemetamol, except for 3 volunteers for which [^{18}F]Florbetapir was used. PET-scans were performed on an ECAT EXACT+ HR scanner (Siemens, Erlangen, Germany). Participants received a single dose of the radioligand in the antecubital vein (target dose 185 MBq); images acquisition started 85min after the injection and consisted of 4 frames of 5 minutes, followed by a 10 minutes transmission scan using ^{68}Ge line sources. Images were reconstructed using filtered back-projection algorithm

including corrections for measured attenuation, dead time, random events, and scatter using standard software (Siemens ECAT – HR + V7.1, Siemens/CTI Knoxville, TN, USA). Individual PET average images were produced using all frames and were then manually reoriented according to Magnetisation Transfer (MT)-weighted structural MRI volumes and coregistered to the individual space structural MT map. Flow-field deformation parameters obtained from DARTEL spatial normalisation of the MT maps were applied to averaged co-registered PET images (Ashburner, 2007). We did not provide correction for partial volume effect, as this type of PET processing was not included in Centiloid scaling pipeline (Klunk et al., 2015). Volumes of interest were determined using the automated anatomical labelling (AAL) atlas (Tzourio-Mazoyer et al., 2002). Standardised uptake value ratio (SUVR) was computed using the whole cerebellum as reference region (Klunk et al., 2015). As images were acquired using 2 different radioligands, their SUVR values were converted into Centiloid Units (W. E. Klunk et al., 2015). A β burden was averaged over a composite mask covering the previously reported earliest aggregation sites for A β pathology (Grothe et al., 2017), that is: frontal medial cortex and basal part of temporal lobe (fusiform & inferior temporal gyri).

Cognitive assessment

A cognitive battery of neuropsychological tasks was carried out in two sessions, while well-rested. A first session of ~1h was performed in the afternoon prior to the sleep assessment, approximately 7.5h before habitual bedtime, and a second session of ~1.5h was performed on another day (between 12 and 6h prior to habitual bedtime). From those two sessions, three domain-specific composites scores were computed for the memory, executive function, and attentional domains, and consisted of the standardised sum of the standardised domain-specific scores, where higher values indicate better performance. A fourth global cognitive score consisted of the standardised sum of the domain-specific composite scores.

The first session and comprised: (1) Mnemonic Similarity Task (MST)(Stark, Yassa, Lacy, & Stark, 2013); (2) Category Verbal Fluency (letter and animals) (Cardebat, Doyon, Puel, Goulet, & Joanette, 1990); (3) Digit Symbol Substitution Task (DSST) (Wechsler, 1997); (4) Visual N-back Task (1, 2 and 3-back variants) (Kirchner, 1958); and (5) Choice Reaction Time (CRT) (Zimmermann & Fimm, 2002). The second session of ~1.5h was performed on another day (between 12 and 6h prior to habitual bedtime) and comprised: (1) Direct and Inverse Digit Span Task (Wechsler, 1997); (2) Free and Cued Selective Reminding Test (FCSRT) (Grober, Buschke, Crystal, Bang, & Dresner, 1988); (3) a computerised version of

the Stroop Test (Stroop, 1935); (4) Trail Making Test (TMT) (Bowie & Harvey, 2006) and (5) D2 Attention Test (Brickenkamp, 1966). The memory score consisted of the FCSRT (sum of all 4 free recalls) and the recognition memory score from the MST. The executive function score included Verbal Fluency tests (letter and animals score for 2min), inverse order digit span, TMT (part B), N-back (3-back variant) and Stroop Test (interfering items errors). The attentional score comprised the DSST, TMT (part A), N-back (1-back variant), D2 (Gz-F) and CRT (reaction time to dissimilar items).

Statistics

Statistical analyses were performed in SAS 9.4 (SAS Institute, Cary, NC) using Generalised Linear Mixed Models (GLMMs). The distribution of dependent variables was determined by fitting all parametric probability distributions to data, using the “allfitdist” function in Matlab 2015 (<http://amir.eng.uci.edu/MvCAT.php>) and GLMMs were adapted accordingly as preconised by SAS statisticians. Subject was treated as a random factor (intercept): each model included sex and age as covariates, as well as education for models with cognitive score as dependent variables. Statistical significance threshold was set at $p < 0.05$ as no correction for multiple comparisons were required. The association between arousals and A β was tested in a single model including arousal density as dependent variable together with transition (T+, T-) and EMG (M+, M-) arousal statuses and early A β burden as regressors (as well as sex and age). The association between arousal and cognition A β was tested in a single model including global cognition score as dependent variable together T+M- and T-M+ arousal density as regressors (as well as sex and age). Kenward-Roger’s correction was used to determine degrees of freedom. 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). P values in post-hoc contrasts (difference of least square means) were adjusted for multiple testing using Tukey’s procedure. Cook’s distance was used to assess the potential presence of outliers driving the associations, and as values ranged below 0.4 no datapoint was excluded from the analyses (a Cooks distant > 1 is typically considered to reflect outlier value).

Study approval

The study was registered with EudraCT 2016-001436-35. All procedures were approved by the Hospital-Faculty Ethic Committee of ULiège. All participants signed an informed consent prior to participating in the study.

Chapter VII: Frontal grey matter microstructure is associated with sleep slow waves characteristics in late midlife

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This chapter is in preparation for publication as a research article.

ABSTRACT

Study objectives. Human ageing is characterised by alterations in sleep-wake regulation that have been directly associated with changes in brain structural integrity. In particular, the ability to generate slow waves (SW) 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 SW, their relationships with cerebral microstructure is not established.

Methods. We recorded habitual sleep under EEG in 99 healthy late-midlife individuals (mean age = 59.3±5.3 years; 65 women) and extracted SW parameters (*i.e.* density, amplitude, frequency). We further used multi-shell diffusion-weighted imaging and neurite orientation dispersion and density imaging (NODDI) to assess microstructural integrity over the frontal cortex grey matter.

Results. In statistical models adjusted for age, sex, and sleep duration, we found that mean SW frequency was positively associated with neurite density in frontal grey matter. In addition, a lower SW density, particularly of fast switcher SW, showing a faster down-to-up frequency, was associated with reduced neurite orientation dispersion. By contrast, we found no significant relationships between SW amplitude and NODDI metrics.

Conclusions. Our findings suggest that frontal cortical neurite density plays a key role for neural synchronisation during sleep, while the complexity of neurite organisation contributes to the rate of SW occurrence. Preserved frontal grey matter microstructure seems therefore crucial for SW generation and their electrophysiological characteristics during NREM sleep in ageing.

Keywords (3-10): Slow waves; Diffusion-weighted imaging; Brain microstructure; Frontal cortex; NODDI; Neurite density; Neurite orientation; ageing

INTRODUCTION

Human ageing is associated with marked changes in the regulation of sleep and wakefulness(Mander et al., 2017), that are detected as early as in the 5th decade of life(Christian Cajochen et al., 2006; Carrier et al., 2011). These changes predict the risk of incident dementia(Shi et al., 2018) and most likely contribute to the increased prevalence of sleep complaints and insomnia disorder reported in ageing(C. M. Morin, LeBlanc, Daley, Gregoire, & Mérette, 2006; Robbins et al., 2021). At the electroencephalographic (EEG) level, various features of sleep slow waves (SW), a gold standard marker of sleep pressure reflecting neuronal synchronisation, are notably affected in older individuals(Léger et al., 2018), as reflected by reduced SW density and amplitude, smoother SW slope, and increased duration of the down and up states(Carrier et al., 2011). These age-related changes are most prominent over the frontal lobe(Carrier et al., 2011), which is particularly involved in the generation of SW(Jonathan Dubé et al., 2015; M. Murphy et al., 2009) and shows high sensitivity to prior sleep-wake history(Schmidt, Peigneux, & Cajochen, 2012). Though SWs are most often considered as a whole, their heterogeneity is now well established(P. Achermann & Borbély, 1997). Recently, work by Bouchard and colleagues proposed a novel criterion to distinguish SW types based on the frequency of the transition from the down to up state, differentiating thus so-called slow and fast switchers(Bouchard et al., 2021). Critically, they found that the decrease in SW density observed in ageing was most prominent for the fast switchers compared with the slow switcher SWs.

Age-related changes in sleep-wake regulation have been associated with widespread alterations in brain macrostructure(Baillet & Schmidt, 2020). Accordingly, several cross-sectional studies indicated relationships between the age-related differences in grey matter (GM) integrity measures, such as cortical volume or thickness, and sleep-wake parameters either as self-reports(Carvalho et al., 2017) or objective EEG(A.-A. Baril et al., 2021; Jonathan Dubé et al., 2015; Van Someren et al., 2018) and actigraphy(Lim et al., 2016) metrics. In longitudinal protocols, these indices have in turn been reported to predict higher rates of GM deterioration(Sexton et al., 2014; Spira et al., 2016), suggesting bidirectional detrimental influences between the alterations in brain macrostructure and impaired sleep quality(Van Egroo, Narbutas, Chylinski, Villar González, Maquet, et al., 2019b).

Recent advances in magnetic resonance imaging (MRI) have provided new means to investigate age-related changes in brain tissue microstructure *in vivo*. Among them, the neurite orientation dispersion and density imaging (NODDI) is a multi-compartment diffusion-weighted model which, by discriminating between diffusion signal originating from intracellular, extracellular, and isotropic diffusion (H. Zhang, Schneider, Wheeler-Kingshott, & Alexander, 2012), enables the investigation of microstructural features within the cortex (Fukutomi et al., 2018; Gozdas, Fingerhut, Dacorro, Bruno, & Hosseini, 2021) thanks to quantitative indices probing neurite density (NDI) and their orientation dispersion (ODI).

Here, we recorded habitual sleep via EEG and acquired NODDI data in a large sample of healthy individuals aged 50 to 69 years ($N=99$; 59.3 ± 5.3 y; 68 women). We computed the average characteristics of the SW produced during NREM sleep over the frontal cortex, including the density, the peak-to-peak amplitude, and frequency. We further extracted grey matter NDI and ODI metrics over a set of frontal regions critically involved in the generation and the propagation of sleep SWs. We hypothesised that higher density and lower orientation dispersion of neurites, indicative of a preserved microstructural integrity, would be associated with preserved SW characteristics, *i.e.* higher density and peak-to-peak amplitude, along with slower frequency.

METHODS

Study design and participants

One hundred and one healthy older individuals (mean age = 59.3 ± 5.3 years; 68 women) were enrolled for this study (**Table 1**) between June, 15th 2016 and October, 2nd 2019 for a multi-modal cross-sectional study taking place at the GIGA-Cyclotron Research Centre/In Vivo Imaging of the University of Liège (Cognitive fitness in ageing – COFITAGE – study; EudraCT: 2016-001436-35). Two participants were excluded from the analyses, due to technical issues with the MRI and sleep data, respectively. All participants were devoid of clinical symptoms of cognitive impairment, as verified by dementia rating scale scores > 130 and mini mental state examination scores > 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 ($BAI \geq 17$) (Aaron T. Beck, Epstein, Brown, & Steer, 1988);

depression, as assessed by the 21-item self-rated Beck Depression Inventory ($BDI \geq 17$) (A. T. Beck, Steer, & G., 1988). Participants with stable treatment (> 6 months) for hypertension and/or hypothyroidism were included in the study. Individuals with sleep apnoea (apnoea-hypopnoea 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.

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

Sex	68 ♀ / 31 ♂
Age (years)	59.3 $\pm$ 5.3 [50-69]
Education (years)	15.2 $\pm$ 3.0 [9 - 25]
Total sleep time (TST) (minutes, EEG)	393.1 $\pm$ 47.0 [229 - 495.5]
Time spent in sleep stage (% of TST, EEG)	
N1	6.2 $\pm$ 2.8 [0.6 – 15.6]
N2	51.3 $\pm$ 8.7 [31.4 - 75.7]
N3	19.1 $\pm$ 6.5 [7.2 - 38.3]
REM	23.3 $\pm$ 6.8 [6.5 - 39.8]
Mean SW density (number/minute of N2/N3)	
All slow waves	7.1 $\pm$ 4.3 [0.8 – 19.2]
Slow switchers	4.9 $\pm$ 3.1 [0.6 - 15.9]
Fast switchers	2.1 $\pm$ 1.6 [0.1 - 8.8]
Mean SW amplitude (μ V)	
All slow waves	101.6 $\pm$ 12.4 [76.0 - 128.2]
Slow switchers	104.7 $\pm$ 13.7 [77.7 - 131.3]
Fast switchers	94.1 $\pm$ 11.0 [73.0 – 130.1]
Mean frequency	
All slow waves	1.2 $\pm$ 0.1 [1.0 - 1.6]

Slow switchers	1.1 ± 0.1 [1.0 - 1.3]
Fast switchers	1.5 ± 0.1 [1.3 - 1.8]
Mean transition frequency	
All slow waves	1.4 ± 0.1 [1.1 - 1.8]
Slow switchers	1.1 ± 0.0 [1.0 - 1.2]
Fast switchers	2.0 ± 0.1 [1.9 - 2.2]
Median ROI values	
NDI	0.51 ± 0.01 [0.46 - 0.53]
ODI	0.44 ± 0.01 [0.38 - 0.47]

Magnetic resonance imaging acquisition

All MRI data were acquired on a 3 Tesla whole-body Magnetom Prisma scanner (Siemens, Erlangen, Germany), with a 64-channel head coil. Diffusion-weighted (DW) data were acquired using a spin-echo echo-planar imaging sequence with a 2mm isotropic spatial resolution. Acquisition parameters included: TR = 7900 ms, TE = 69 ms, 70 transverse slices, slice thickness = 2 mm, in-plane resolution 2x2 mm² (field of view = 192x216 mm², matrix = 96x106) and acceleration factor 2, bandwidth per pixel = 2170 Hz/Px. The multi-shell DW imaging (DWI) scheme included 118 volumes. The first volume was discarded to avoid T1 saturation effect. The remaining 117 volumes correspond to a total of 105 DW images interleaved with 12 b=0 images. The set of diffusion directions was created using electrostatic repulsion (Slater et al., 2019) and is defined over three shells (b = 650, 1000 & 2000 s/mm²). Five additional b = 0 volumes with identical acquisition parameters but inverted phase encoding direction were acquired for the estimation of susceptibility-induced distortions. In addition to DW data, a structural quantitative multi-parameter protocol (1mm³ voxel size) was acquired and used to generate magnetisation transfer saturation (MTsat) maps as previously described (Van Egroo et al., 2021) for subsequent data segmentation and normalisation.

Magnetic resonance imaging processing

The MRI processing pipeline is summarised and illustrated in **Supplementary Fig. S1**. For each individual, the MTsat map was segmented into tissue class maps of GM, white matter (WM), and cerebrospinal fluid (CSF) posterior probability using Unified

Segmentation(Ashburner & Friston, 2005). The segmentation outputs were then diffeomorphically registered to a study-specific template created with DARTEL(Ashburner, 2007), and normalised to the Montreal Neurological Institute (MNI) space using DARTEL-estimated individual deformation fields. For DW data, susceptibility-induced and eddy current-induced distortions, as well as participant's movements, were first estimated and corrected using *topup*(Andersson, Skare, & Ashburner, 2003) and *eddy*(Andersson & Sotiropoulos, 2016) in FSL 5.0.9. Whole-brain NODDI parameter maps (NDI, ODI) were then computed using the Microstructure Diffusion Toolbox(R.L. Harms, Fritz, Tobisch, Goebel, & Roebroeck, 2017) (v1.2.6, <https://github.com/robbert-harms/MDT>). The NDI and ODI maps were registered to the MTsat map and brought into the MNI space by applying normalisation parameters estimated previously. Smoothing of normalised NODDI maps was further performed 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.

The region of interest (ROI) was defined as a set of frontal regions commonly involved in studies of sleep SW(Dang-Vu, 2012; Dang-Vu et al., 2005; Mander et al., 2015, 2013; Saletin, van der Helm, & Walker, 2013). Based on regions from the Automated Anatomical Labelling atlas (AAL2)(Tzourio-Mazoyer et al., 2002), we built a composite mask including, bilaterally, medial prefrontal, medial orbito-frontal, dorsolateral frontal, insula, and anterior cingulate cortices. For each individual, median regional NDI and ODI signals were obtained by applying the ROI on the normalised GM-weighted smoothed NODDI maps. While not completely eliminating potential partial volume effects, this GM-specific processing pipeline aimed at investigating cortical microstructure after removing unwanted contributions from WM and/or CSF to diffusion signal in each voxel.

Sleep EEG acquisition and processing

As part of the screening process, participants first performed an in-lab adaptation and screening night to minimise the disrupting effect caused by sleeping in a novel environment(Tamaki et al., 2016). 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 naps, as well as 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. 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. The signal was re-referenced to the average of both mastoids. Scoring of baseline night in 30-s epochs was performed automatically using a validated algorithm (ASEEGA, PHYSIP, Paris, France)(Berthomier et al., 2007; L Peter-Derex et al., 2020). An automatic detection algorithm with adapting thresholds was further applied to exclude artefacts and arousals from the scored data(Chylinski et al., 2020; Dorothee. Coppieters 't Wallant et al., 2016)

SWs were automatically detected on N2 and N3 epochs free of artefacts > 5s, via a previously developed algorithm(Thaïna Rosinvil et al., 2021). First, data were band-filtered between 0.3 and 4 Hz with a linear phase finite impulse response filter. Based on recent work(T Rosinvil et al., 2020), the SW detection criteria were adapted for age and sex(Thaïna Rosinvil et al., 2021): instead of the standard $\geq 75\mu\text{V}$ peak-to-peak amplitude and $\geq -40\mu\text{V}$ for the negative peak, the thresholds were lowered to $\geq 70\mu\text{V}$ and $\geq -37\mu\text{V}$ for women, and $\geq 60.5\mu\text{V}$ and $\geq -32\mu\text{V}$ for men. The duration of the negative deflection ranged between 125 and 1500ms, and the duration of the positive deflection had to fit below 1000ms. Over the frontal electrodes (F3-Fz-F4), for all SWs corresponding to the aforementioned criteria, the following parameters were extracted: SW density, computed as the number of SW per minute of N2/N3 sleep; SW amplitude, corresponding to the amplitude in μV from the down to the up state; and SW frequency, corresponding to the inverse of the total duration of the SW. After being computed for each of the three frontal electrodes separately, those parameters were then averaged; it is the frontal average of these parameters which was used in the statistical models reported below. As described elsewhere(Bouchard et al., 2021), SW were sorted according to their down-to-up state transition frequency into either slow ($<1.2\text{Hz}$) or fast switchers ($\geq 1.2\text{Hz}$) SWs. For exploratory analyses, SW parameters (SW density, amplitude, and frequency) were also computed for those two types of SWs separately.

Statistics

Statistical analyses were performed using Generalised Linear Mixed Models (GLMMs) in SAS 9.4 (SAS Institute, Cary, NC). For each statistical model, the distribution of the dependent variable distribution was first determined in MATLAB and the GLMMs were

adjusted accordingly. All GLMMs with SW characteristics as dependent variable included age, sex, as well as total sleep time (TST) as covariates. 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, Edwards, Das, & Sen, 2017a).

RESULTS

We first assessed the effect of age on both NODDI parameters over the frontal ROI using GLMMs adjusted for sex. We found that NDI was not significantly associated with age ($F_{1,96} = 1.64$, $p = 0.2$), or sex ($F_{1,96} = 2.2$, $p = 0.14$). ODI, on the other hand, showed a significant, negative relationship with age ($F_{1,96} = 14.89$, $p = 0.0002$), but not with sex ($F_{1,96} = 1.58$, $p = 0.21$) (**Figure 1**).

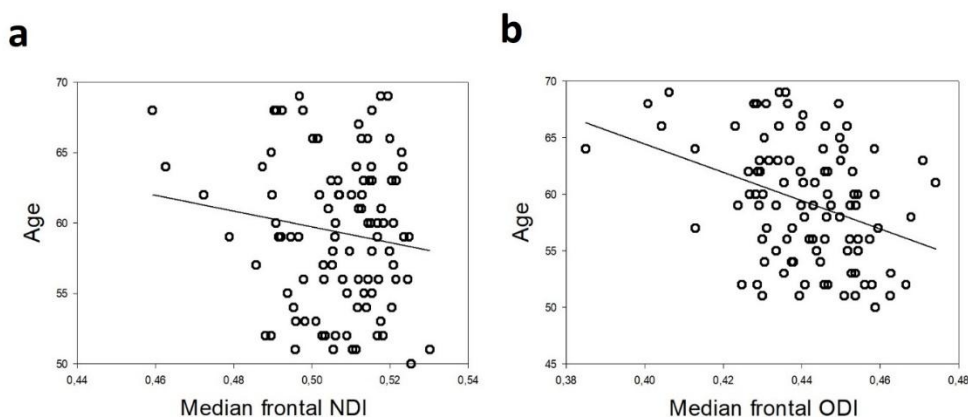


Figure 1. Association between the median frontal NDI (panel A) and median frontal ODI (panel B) and the age of participants. Simple regressions were used only for a visual display and do not substitute the GLMM outputs.

Neurite density (NDI)

We assessed whether SW density was associated with NDI in the frontal ROI (**Figure 2A**), while controlling for age, sex and TST. We found no significant association with median NDI values ($F_{1,94} = 1.51$, $p = 0.22$), but, in line with the literature (Carrier et al., 2011), a

significant negative link with age, as well as an effect of sex, with lower SW density in men. A similar pattern was observed for SW amplitude, for which we found no significant association with median NDI values ($F_{1,94} = 1.36$, $p = 0.25$), but an expected (T Rosinvil et al., 2020) significant negative relationship with age, and sex with, again, lower values in men. By contrast, a GLMM with the SW frequency as dependent variable revealed a significant positive association with median NDI values ($F_{1,94} = 4.64$, $p = 0.03$, $R^2_{\beta^*} = 0.05$, **Figure 2B**), but no significant effect of age, sex, or TST. Given this result, we aimed to explore if this relationship was driven by a specific type of SW (slow or fast switchers), but we found no significant relationship between the total frequency of a specific SW type and NDI values (slow switchers: $F_{1,94} = 3.22$, $p = 0.07$; fast switchers $F_{1,94} = 2.12$, $p = 0.15$). Detailed outputs of all the GLMMs relating SW characteristics to frontal NDI values are summarised in **Table 2**.

Table 2. Outputs of GLMMs performed on NDI values (one line represents one model).

	Age	Sex	TST	NDI
SW density	F = 4.88 p = 0.030 $R^2_{\beta^*} = 0.05$	F = 10.57 p = 0.0016 $R^2_{\beta^*} = 0.10$	F = 0.32 p = 0.58	F = 1.51 p = 0.22
SW amplitude	F = 9.77 p = 0.002 $R^2_{\beta^*} = 0.09$	F = 138.51 p < 0.0001 $R^2_{\beta^*} = 0.60$	F = 3.48 p = 0.07	F = 1.36 p = 0.25
SW frequency	F = 1.01 p = 0.32	F = 2.53 p = 0.12	F = 0.23 p = 0.64	F = 4.64 p = 0.03 $R^2_{\beta^*} = 0.05$
Slow switchers frequency	F = 0.32 p = 0.57	F = 1.49 p = 0.23	F = 0.75 p = 0.39	F = 3.22 p = 0.076
Fast switchers frequency	F = 1.40 p = 0.24	F = 4.64 p = 0.03 $R^2_{\beta^*} = 0.05$	F = 0.03 p = 0.85	F = 2.12 p = 0.15

Degrees of freedom for all the aforementioned models are 1 (numerator) and 94 (denominator)

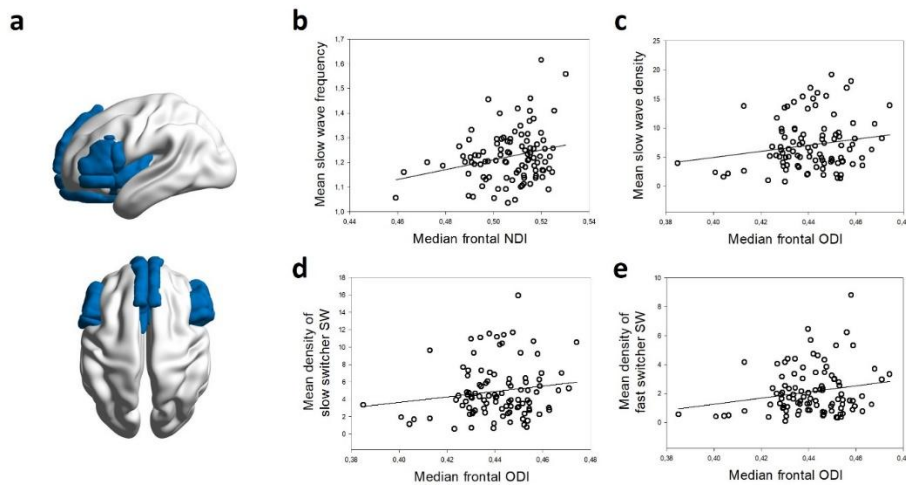


Figure 2. Relationships between slow wave characteristics and frontal ROI NDI and ODI metrics. NODDI metrics were obtained for a set of frontal regions shown in blue (panel A). Positive association between the NDI and mean slow wave frequency (panel B). Positive association between the ODI and mean slow wave density (panel C). No significant association between the ODI and slow switcher SW density (panel D). Positive association between the ODI and fast switcher SW density (panel E). Simple regressions were used only for a visual display and do not substitute the GLMM outputs.

Neurite orientation dispersion (ODI)

In a second set of GLMMs, we aimed to assess the associations between SW characteristics and the ODI metric. We found a positive relationship between median ODI values and SW density, although as a statistical trend ($F_{1,94} = 3.75$, $p = 0.056$, $R^2_{\beta^*} = 0.04$, **Figure 2C**), on top of the sex effect, but no effect of age or TST was found (Table 3). Given this borderline result, we examined if the median ODI values would be selectively associated with a specific type of SW (slow or fast switchers). We found a significant association between the density of fast switcher SW and median ODI values ($F_{1,94} = 5.82$, $p = 0.018$, $R^2_{\beta^*} = 0.06$, **Figure 2E**), but not for the density of slow switcher SW ($F_{1,94} = 2.36$, $p = 0.13$) (**Figure 2D**). In a GLMM with SW amplitude as the dependent variable, we observed no link with median ODI values ($F_{1,94} = 0.23$, $p = 0.64$), on top of an effect of age and sex, but not of TST. Regarding the SW frequency, we found no significant relationship ($F_{1,94} = 0.94$, $p = 0.33$), and no effect of age, sex or TST. The detailed outputs of all the GLMMs relating the SW characteristics to the frontal ODI values are summarised in **Table 3**.

Table 3. Outputs of GLMMs performed on ODI values (one line represents one model).

	Age	Sex	TST	ODI
SW density	F = 2.41 p = 0.12	F = 13.39 p = 0.0004 R²_β* = 0.12	F = 0.68 p = 0.41	F = 3.75 p = 0.056 R²_β* = 0.04
Slow switchers density	F = 1.83 p = 0.18	F = 9.31 p = 0.003 R²_β* = 0.09	F = 0.37 p = 0.55	F = 2.36 p = 0.13
Fast switchers density	F = 2.88 p = 0.09	F = 18.59 p < 0.0001 R²_β* = 0.17	F = 1.05 p = 0.31	F = 5.82 p = 0.018 R²_β* = 0.06
SW amplitude	F = 8.62 p < 0.0001 R²_β* = 0.08	F = 148.93 p < 0.0001 R²_β* = 0.61	F = 2.62 p = 0.11	F = 0.23 p = 0.64
SW frequency	F = 0.52 p = 0.47	F = 3.69 p = 0.06	F = 0.00 p = 0.99	F = 0.94 p = 0.33

Degrees of freedom for all the aforementioned models are 1 (numerator) and 94 (denominator)

DISCUSSION

Physiological ageing 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, et al., 2019b). Expanding on previous studies which focused on macrostructural measures of brain structural integrity to investigate the neurobiological correlates of the age-related decrease in NREM sleep SW generation (A.-A. Baril et al., 2021; Jonathan Dubé et al., 2015; Mander et al., 2013; Saletin et al., 2013), we tested whether brain tissue microstructural properties as assessed *in vivo* with the NODDI biophysical model could be associated with SW characteristics in a sample of healthy late midlife individuals aged 50

to 69y. We first found that a higher frequency of the SW was associated with a higher neurite density over a frontal cortex ROI. Moreover, we report that a lower density of SW over the same ROI, specifically of the fast switcher SW, was linked to a lower neurite orientation dispersion.

The changes in neuromodulator concentrations associated with NREM sleep lead the neurons to spontaneously alternate between down and silent, hyperpolarised and up and firing, depolarised periods (F. Ding et al., 2016; M. Steriade, Timofeev, & Grenier, 2001)[REF]. These alternations are synchronized across patches of the cortical tissues generating an electrical signal taking the form of a SW on the scalp EEG. The number of neurons contributing to a SW and their relative synchrony will determine the amplitude of the detected SW as well as the duration of the SW up and down states that will determine the total frequency of the SWs. This will in turn determine in part the density of the SWs as only those brain oscillations reaching a given amplitude will be considered as a SW (Iber et al., 2007; T Rosinvil et al., 2020). The density of SWs will also depend on the need for them, which, according to a wealth of literature, reflects the need for sleep(Vladyslav V. Vyazovski, Cirelli, & Tononi, 2011). Finally, the synchrony of neuron transition from the down to the up state will mainly drive the transition frequency of a SW (Bouchard et al., 2021).

The age-related decrease in the frequency of the SW has been speculated to be the consequence of less synchronicity between cortical neurons when entering the hyperpolarised and depolarised states (Bouchard et al., 2021; Carrier et al., 2011). We find that the density of neurites, as indexed by the NDI values, was positively associated with the mean frequency of the SWs, beyond the effect age can have on the latter. This could mean that in healthy late midlife, more neurites tighten the bounds between neurons and facilitates the synchrony of entering synchronously enter up and down states. This interpretation is reinforced by the fact that the transition frequency of the SWs is also significantly positively linked with the neurite density index. Our finding support therefore that the age related decrease in the SW transition frequency leading to a more prominent loss of the fast switcher SWs during aging(Bouchard et al., 2021) is triggered, at least in part, by a relative loss of neurites within the cortical mantle. Our study does not indicate, at least not in healthy and relatively young older adults, that the density of neurites is linked to the density of the SWs. This suggest that the mere number of neurite synaptic connections within the grey matter tissue is not related to the need for sleep, at least much less so than to the synchrony of SWs.

The ODI values reflect the coherence of neurites orientation within the underlying tissue, a low index indicating completely aligned fibres, while a high index indicates isotropy(Kamiya, Hori, & Aoki, 2020) (i.e. no dominant orientation). In the grey matter, the ODI thus represents the branching complexity of the dendritic trees and can be seen as a marker of grey matter complexity, with higher isotropy meaning more complexity(H. Zhang et al., 2012). Despite the limited age-range of our sample, we find that the ODI index decreases as one gets from 50 to 70y. This suggests that preserved brain integrity is associated with a higher neurite orientation dispersion. In line with this inference, we find a positive association between the ODI and SW density, specifically for the density of fast switchers SW(Bouchard et al., 2021). Our results therefore suggest that microstructural grey matter complexity may help maintaining the generation of sleep SW in the sixth and seventh decades of life, particularly in their most fragile form. The complexity of dendritic structures may be related to neuron synchronisation, e.g. by interconnecting a large population of neurons, resulting in a higher density of SWs. Alternatively, the complexity of neurite orientation could be related to a high average synaptic cost that would contribute to a higher need for sleep leading to more SWs. Given the age-related increase in sleep complaints and insomnia disorders(C. M. Morin et al., 2006; Robbins et al., 2021), one could speculate that a reduction in the orientation dispersion of neurite contribute to a less intense and more fragile sleep characterised by less SWs.

Our study includes a relatively large sample of thoroughly screened healthy late midlife individuals. This means that compared to the general population, the variability in the NODDI and SWs parameters was likely reduced in our sample. Yet, we were still able to isolate significant associations between the brain microstructure indices and SWs characteristics. The fact that the brain microstructure inferences we computed were based on state-of-the-art data acquisition and processing approaches(Robbert L. Harms & Roebroek, 2018; Tabelow et al., 2019) most probably contribute to isolating the effects. The limited variability could however only yield small statistical effects size, warranting replication in larger or more diverse samples. Moreover, whereas the literature strongly supports the involvement of frontal regions in age-related changes, both with regards to the structural integrity of the brain and to sleep SWs, the regional aspect of the relationships highlighted here should be extended to the whole brain. As deep pyramidal cells provide most of the recorded EEG signal at the electrode level (M. X. Cohen, 2017) and seem to play an important role in the sleep homeostasis(Krone et al., 2021), characterising NODDI parameters within cortical

layers would refine our understanding of the complex link between microstructural integrity and synchronized activity among neuronal populations, but the spatial resolution inherent to currently available DWI sequences constitutes an important obstacle to do so *in vivo*. It would also be of prime interest to conduct a longitudinal study to assess more thoroughly whether the preservation of the individual brain microstructure is related to the maintenance of SWs characteristics.

An increasing body of evidence shows that the quality of sleep and wakefulness regulation is of prime importance to sustain cognition in normal and pathological ageing (Djonlagic et al., 2020; Latreille et al., 2019; Shi et al., 2018). The quantification of NREM sleep SWs has been associated with higher amyloid-beta ($A\beta$) burden in the medial prefrontal cortex (Mander et al., 2015), a hallmark of Alzheimer's disease (AD) neuropathology, while experimental reduction of sleep SW disturb $A\beta$ content in the CSF (Y. S. Ju et al., 2017). It is therefore tempting to speculate based on the current findings that a preserved neurite content would protect against neuropathological AD-related processes through its positive impact on sleep SWs, and would ultimately constitute a positive factor in successful cognitive ageing. A first step to test this speculation would be to assess whether regional NODDI parameters are associated with regional $A\beta$ burden, and whether it could be linked to cognition in older individuals.

In summary, our cross-sectional study shows that, in healthy late midlife, brain microstructural aspects related to neurite density and orientation dispersion, as measured over the frontal lobe using DWI approaches, are associated with the preserved ability or need to generate SWs during NREM and do so through synchronous neuronal activity. These findings may have implications for successful ageing, insomnia and neurodegenerative diseases.

Chapter VIII: Timely sleep coupling: spindle-slow wave synchrony is linked to early amyloid- β burden and predicts memory decline

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This chapter is in preparation for publication as a research article.

ABSTRACT

Sleep alteration is a hallmark of ageing and emerges as a risk factor for Alzheimer's disease (AD). While the fine-tuned coalescence of sleep microstructure elements may influence age-related cognitive trajectories, its association with AD-processes is not fully established. Here, we investigated whether the coupling of spindles and slow waves is associated with early amyloid-beta ($A\beta$) brain burden, hallmark of AD neuropathology, and cognitive change over 2 years in 100 late-midlife healthy individuals (50-70y; 68 women). We found that, in contrast to other sleep metrics, earlier occurrence of spindles on slow-depolarisation slow waves is associated with higher medial prefrontal cortex $A\beta$ burden ($p=0.014$, $r^2_{\beta*}=0.06$), and is predictive of higher longitudinal memory decline ($p=0.032$, $r^2_{\beta*}=0.07$). These findings unravel early links between sleep, AD-related processes and cognition and suggest that altered coupling of sleep microstructure elements, key to its mnemonic function, contributes to poorer brain and cognitive trajectories in ageing.

INTRODUCTION

Alterations in sleep quality are typical of the aging process with a more fragmented and less intense (or shallower) sleep detected as early as the fifth decade of life (Carrier et al., 2011). Beyond healthy ageing, alterations in sleep are predictive of the risk of developing Alzheimer's disease (AD) over the next 5 to 10 years (Lim et al., 2013; Musiek & Holtzman, 2016). Similarly, sleep disorders such as insomnia and obstructive sleep apnoea syndrome are associated with increased odds for AD diagnosis (A. A. Baril et al., 2018; Elias et al., 2018). Brain burdens of aggregated amyloid- β ($A\beta$) and tau proteins, hallmarks of AD pathophysiology, have been linked with a reduced sleep intensity, as indexed by the overall production of slow waves during sleep, but also to worse objective sleep efficiency and subjective sleep quality, in healthy and cognitively normal older individuals aged $> 70y$ (Ju et al., 2013; Ju et al., 2017; Kang et al., 2009; Lucey et al., 2019; Mander et al., 2015; Sprecher et al., 2017). Sleep alteration may in turn contribute to the aggregation of AD proteins: experimental sleep deprivation and sleep fragmentation (disturbance in the production of slow wave during sleep) lead to increased concentration of $A\beta$ in the cerebrospinal fluid (CSF), both in animal models and in healthy human populations (Ju et al., 2017; Kang et al., 2009; Lucey et al., 2018; Ooms et al., 2014). Overall, a bidirectional detrimental relationship between sleep quality and the neuropathology of AD is emerging in the literature. Sleep may therefore constitute a modifiable risk factor which one could act upon to prevent or delay the neuropathological processes associated to AD and favour successful cognitive trajectories over the lifespan (Ju et al., 2014; Van Egroo, Narbutas, Chylinski, Villar González, Maquet, et al., 2019b; Wang & Holtzman, 2019). Hitherto, however, sleep is not yet widely recognised as an independent risk factor for AD and the mechanistic associations between sleep and early AD neuropathology are not yet fully established.

Sleep microstructure elements, such as sleep spindles and slow waves (SW), are essential correlates of cognitive function of sleep, as higher densities of both elements during post-learning sleep have been linked to a better overnight memory consolidation (Gais et al., 2002; Mednick et al., 2013; Miyamoto et al., 2017; Schmidt et al., 2006; Ulrich, 2016). Oddly enough, SW activity (i.e. a power measure combining the density and amplitude of sleep SW over sleep cycles in the 0.75 to 4 Hz band) was reported to modulate the regression between prefrontal cortex $A\beta$ burden and a lower overnight memory consolidation in cognitively normal older individuals (Mander et al., 2015). The fine-tuned coupling of spindles and SW has further been reported to be altered in ageing, with an earlier occurrence of the spindle relative to the SW depolarisation phase in the older compared to younger individuals, and to

predict overnight memory retention (Helfrich et al., 2018). Whether this spindle-SW coupling in ageing is associated to AD-pathological processes and cognitive trajectories is not fully established, however. A study in 31 individuals, aged around 75y, found a link between the brain deposit of tau protein and the coupling of spindles and SW (Winer et al., 2019). By contrast, another research failed to find a link between this coupling and A β brain burden (Winer et al., 2020). Here, we argue that, on top of potential statistical power issues, the difficulty to detect this link may be due to the fact that the assessments were carried out late over the lifespan (i.e. > 70y), when subtle linear associations may be masked by concurrent brain alterations, and by the heterogeneity of sleep SW.

The low frequency oscillations of the electroencephalography (EEG) have been divided in slow oscillations (≤ 1 Hz) and delta waves (1-4 Hz) for decades in humans, notably based on the theoretical framework of the generation of SW (Achermann & Borbély, 1997; Lee et al., 2004). As one of the main features of the SW resides in their transition from down-to up-state, reflecting synchronised depolarisation, a recent work proposed the transition frequency of the down-to-up state as a way of distinguishing between *slow* and *fast* switcher SWs (Bouchard et al., 2021). Compared to young adults, on top of exhibiting a typical overall lower density of SW, older individuals reportedly exhibit higher probabilities of producing slow compared to fast switcher SWs.

Investigating the coupling of spindles and SW, appropriately split between the slow and fast switchers, in late middle-aged healthy adults may be the best approach to gain insight into the biology underlying the early relationship between sleep and AD-related processes. In a longitudinal study, we therefore tested whether the coupling of spindles with the slow and the fast switcher SWs is associated with the early brain burden of A β and memory performance in a large sample (N=100) of healthy and cognitively normal individuals in late midlife (50-70y). We recorded habitual sleep in those individuals devoid of sleep disorders of both sexes (59.5 $\pm$ 5y; 68 woman) under EEG and extracted the density and coupling of spindle and fast and slow switcher SWs over frontal derivations. The burden of A β was assessed using Positron Emission Tomography (PET) tracers ($^{[18F]}$ Flutemetamol, N=96; $^{[18F]}$ Florbetapir, N=4) over the medial prefrontal areas, known to be an early site for A β deposits and the most important generator of SWs during sleep (Dang-Vu et al., 2005, 2010; Mander et al., 2015; Saletin et al., 2013). Performance to the Mnemonic Similarity Task (MST), a memory task highly sensitive to early signs of cognitive decline (Marks, Lockhart, Baker, & Jagust, 2017; Stark et al., 2013), was assessed in all participants concomitantly to EEG and PET measurements (N=100) as well as at follow-up, 2 years later, in a substantial subsample (N=67). We hypothesised that our

large sample of individuals, positioned relatively early in the ageing process, would allow to detect subtle associations between the impaired fine-tuned coupling of spindles and slow and fast switcher SWs, and both the early A β burden and memory performance decline over 2 years.

RESULTS

Slow but not fast switcher SW show preferential coupling with spindles

We first assessed whether spindles were specifically associated with a particular phase on the down-to-up state transition of the SWs, considering the two types of SWs (slow and fast switchers). Of the 341 836 detected slow switcher SWs, 75 910 co-occurred with a spindle (22%); while of the 78 235 fast switcher SWs, 26 912 (34%) were found to co-occur with a spindle. Regarding spindles, 563 928 spindles were detected over all the recordings, of which 102 822 were coupled to a SW (18%), 75 910 to slow switcher SWs (13%) and 26 912 to fast switcher SWs (5%). Statistical analysis with Watson's U^2 test showed that the distribution of spindle onset was significantly different between slow and fast switcher SWs (**Figure 1B**) ($U^2 = 71.143$, $p < 0.001$). Indeed, spindles were preferentially anchored only onto slow switchers, as spindles were most often initiated on the ascending phase of the depolarised state of the slow switcher SWs, while no such preferred coupling was detected for fast switcher SWs (**Figure 1C**). As the slow switcher SWs are the only SW to exhibit a preferential coupling with spindles, they remained our main focus of interest for the remaining analyses.

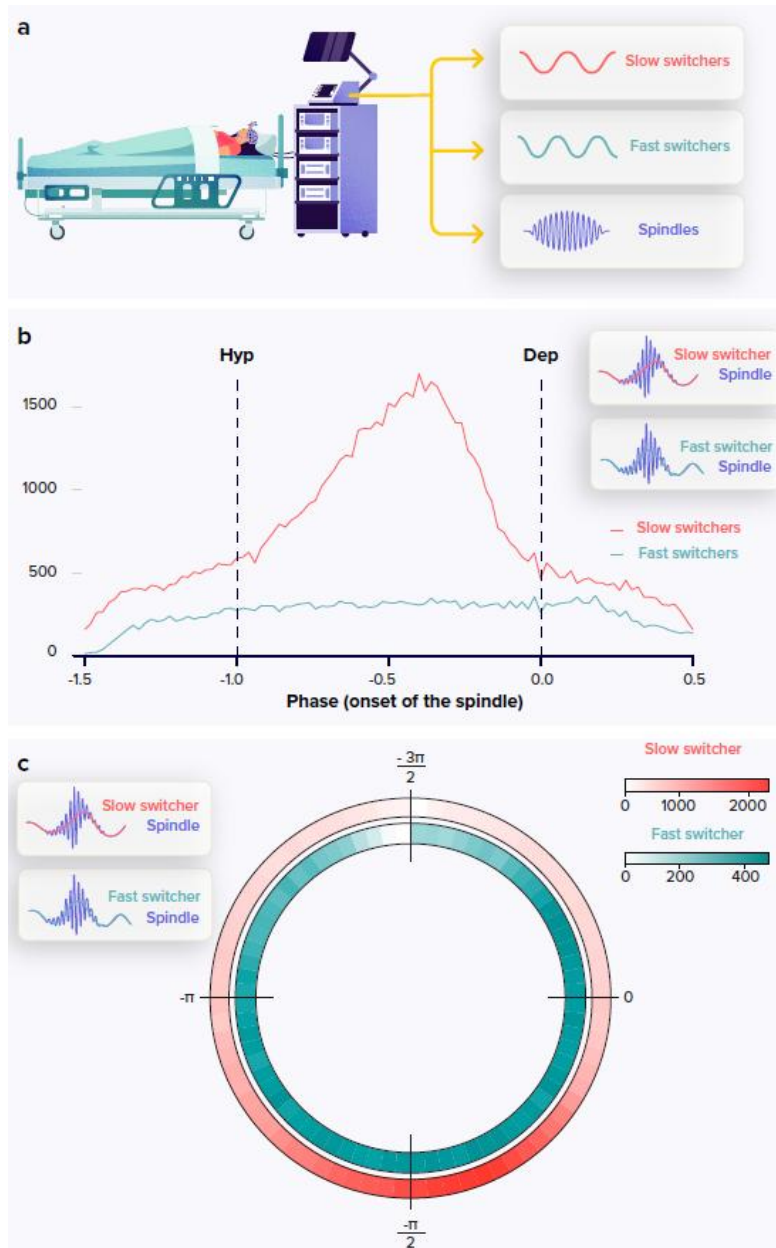


Figure 1. Following a screening night and a regular sleep-wake schedule for 1 week, the participants ($N=100$; age $\pm$; 68 women) slept in the lab at their habitual times under EEG recording. We extracted the density and coupling of spindle and fast and slow switcher SWs over frontal derivations during N2 and N3 sleep stage from EEG recordings (panel A). Analysis of the anchoring of the spindles onto the SW showed a preferential coupling phase only for slow (red) but not fast switcher SWs (light blue) (panel B). Circular representation of the anchoring of the spindles onto the SW phase: $-\pi/2$ represents the hyperpolarisation (down state), 0 represents the depolarisation of the SW. Heatmap represents the

density of the spindles with their onset on specific slow wave phase in 5° bins across all participant nights, for slow (red) and fast switcher SWs (light blue)(panel C).

Spindle onset on slow switcher SW is linked to prefrontal A β burden

Statistical analysis revealed that the anchoring of the spindle onset onto slow switcher SWs was significantly linked to the burden of A β over the medial prefrontal cortex (MPFC) (**Figure 2A**) (main effect of A β PET uptake: $F_{1,96}=6.2$, $p=0.014$, $r^2_{\beta^*}=0.06$), where earlier onset of the spindle relative to the SW phase was associated with higher A β PET uptake (**Figure 2B**). This effect was detected while controlling for the differences between sexes (main effect of sex: $F_{1,96}=5.01$, $p=0.028$), as a later spindle onset was found in men relative to women, and controlling for age (main effect of age, $F_{1,96}=0.04$, $p=0.85$). We assessed the specificity of this association and show that the anchoring of the spindle onset onto fast switcher SWs was not linked to the A β burden over the MPFC (main effect of A β PET uptake: $F_{1,96}=0.20$, $p=0.65$), after controlling for age (main effect of age: $F_{1,96}=0.41$, $p=0.53$) and sex (main effect of sex: $F_{1,96}=0.48$, $p=0.49$) (**Figure 2C**). In addition, the density of slow switcher SWs (main effect of A β PET uptake: $F_{1,96}=0.14$, $p=0.71$) or of spindles (main effect of A β PET uptake: $F_{1,96}=0.09$, $p=0.76$) was not associated with the MPFC A β burden (**Figure 2D-E**), further reinforcing the idea that it is the coupling of sleep microstructure elements that matter rather than their individual occurrence. Likewise, unlike a previous report (Mander et al., 2015), we did not find any association between the MPFC A β burden and several characteristics of the SWs (SWs density - per min of NREM sleep - $F_{1,95}=1.18$, $p=0.28$; spindle density - per min of NREM sleep - $F_{1,95}=0.06$, $p=0.80$; cumulated power generated in the delta band - or slow wave energy (SWE) - $F_{1,95}=0.84$, $p=0.36$; the proportion of slower oscillations (0.5-1 Hz) over the delta power (1.25-4 Hz) - SO/delta proportion - $F_{1,95}=2.82$, $p=0.10$), after correcting for age, sex and total sleep time (SW density: age: $F_{1,95}=5.49$, $p=0.02$, sex: $F_{1,95}=10.26$, $p=0.002$, TST: $F_{1,95}=0.5$, $p=0.48$; spindle density: age: $F_{1,95}=0.56$, $p=0.46$, sex: $F_{1,95}=0.42$, $p=0.52$, TST: $F_{1,95}=0.36$, $p=0.55$; SWE: age: $F_{1,95}=4.2$, $p=0.04$; sex: $F_{1,95}=6.63$, $p=0.01$; TST: $F_{1,95}=0.9$, $p=0.34$; SO/delta proportion: age: $F_{1,95}=3.28$, $p=0.07$, sex: $F_{1,95}=0.0$, $p=0.96$, TST: $F_{1,95}=2.7$, $p=0.10$) (**Suppl. Fig. 1**).

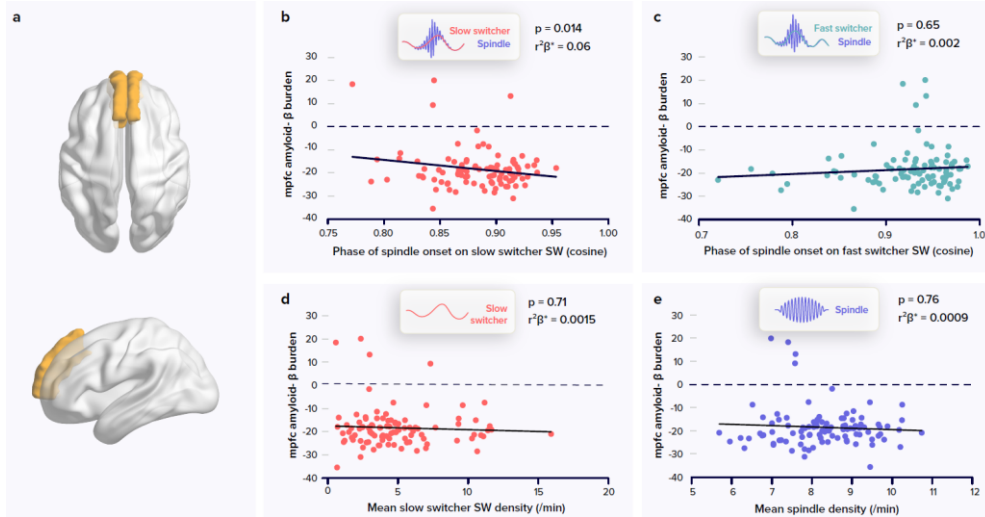


Figure 2. Relationships between spindles and slow waves metrics and the amyloid- β ($A\beta$) burden. PET signal uptake was measured over the medial prefrontal cortex (MPFC) depicted in yellow (panel A). Significant negative association between the MPFC $A\beta$ burden and spindle-slow switcher SW coupling (panel B). No association between the MPFC $A\beta$ burden and spindle-fast switcher SW coupling (panel C). No association between the MPFC $A\beta$ burden and slow switcher SW density (panel D). No association between the MPFC $A\beta$ burden and spindle density (panel E). P values and $r^2\beta^*$ were computed from the GLMMs referred to in the text. Simple regressions were used only for a visual display and do not substitute the GLMM outputs. We used the cosine value of the phase of coupling in the GLMMs (see methods).

To test the apparent difference in the association between the coupling of spindles onto slow and fast switcher SW with the accumulation of $A\beta$ protein, we further computed a statistical model with SW-spindle coupling as the dependent variable, while including the SW type together with the $A\beta$ MPFC burden as independent variables. Statistical analysis yielded a significant interaction between the burden of $A\beta$ in the MPFC and the type of SWs ($A\beta$ burden by SW type interaction: $F_{1,96}=7.05$, $p=0.009$), while *post-hoc* tests indicated that the link between the coupling of the spindle onto the SW and the MPFC $A\beta$ burden was significant for the slow switcher type ($t_{149,8}=-2.00$, $p=0.047$) and not for the fast switcher type ($t_{149,8}=0.56$, $p=0.57$). This finding reinforces the idea that slow and fast switcher SWs constitute distinct realisations of NREM oscillations that are differently associated with brain aggregation of $A\beta$ during the ageing process. This has likely contributed to previous failures to detect links between the coupling of spindles and SW and the deposit of $A\beta$. In fact, when testing the association between the coupling of spindles and SWs, irrespective of the type of SWs, and

PET A β burden over the MPFC, the statistical analysis only yields a weak negative association between the phase of the coupling of the spindle onto the SW and A β burden (main effect of A β uptake: $F_{1,96}=3.96$, $p=0.049$, $r^2_{\beta^*}=0.04$; main effect of sex: $F_{1,96}=4.33$, $p=0.04$; main effect of age: $F_{1,96}=0.05$, $p=0.83$), which could arguably go undetected in a smaller or different sample.

Slow switchers spindle phase coupling predicts memory decline over two years

We tested whether the coupling of spindles with slow switcher SWs was associated with memory performance and its decline over 2 years using the mnemonic similarity task (MST) (**Figure 3A**). The MST consists in a pattern separation task targetting the ability to distinguish between highly resembling memory events, a hippocampus dependent task which is very sensitive to early cognitive decline (Marks et al., 2017; Stark et al., 2013). We found no significant link between the coupling of the spindles onto both SW types and the performance on the task at baseline (i.e. assessed at the same time as the sleep measures) (slow switchers: *main effect of spindle-SW coupling*: $F_{1,96}=0.26$, $p=0.61$; main effect of age: $F_{1,96}=0.36$, $p=0.55$, main effect of sex: $F_{1,96}=0.41$, $p=0.52$, main effect of education: $F_{1,96}=0.47$, $p=0.50$; fast switchers: *main effect of spindle-SW coupling*: $F_{1,96}=0.02$, $p=0.88$; main effect of age: $F_{1,96}=0.34$, $p=0.56$, main effect of sex: $F_{1,96}=0.54$, $p=0.46$, main effect of education: $F_{1,96}=0.40$, $p=0.53$) (**Figure 3B-C**). By contrast, statistical analyses revealed a significant negative link between the relative change in memory performance and the phase of spindle anchoring onto slow switcher SWs, indicating that an earlier spindle onset is predictive of a memory worsening over two years (main effect of spindle-slow switcher SW coupling: $F_{1,61}=4.80$, $p=0.032$, $r^2_{\beta^*}=0.07$), after correcting for age (main effect of age: $F_{1,61}=0.25$, $p=0.62$), sex (main effect of sex: $F_{1,61}=0.20$, $p=0.66$) and education (main effect of education: $F_{1,61}=0.25$, $p=0.62$) (**Figure 3D-E**). No such association was detected when considering spindle coupling to fast switcher SWs (main effect of spindle-fast switcher SW coupling: $F_{1,61}=2.51$, $p=0.12$; main effect of age: $F_{1,61}=0.33$, $p=0.57$, main effect of sex: $F_{1,61}=0.18$, $p=0.68$, main effect of education: $F_{1,61}=1.11$, $p=0.30$). Further statistical analyses show that the memory performance change over the 2-year is not significantly related to the MPFC A β burden (main effect of A β burden: $F_{1,60}=2.33$, $p=0.13$; main effect of age: $F_{1,60}=1.27$, $p=0.26$, main effect of sex: $F_{1,60}=0.03$, $p=0.87$, main effect of education: $F_{1,60}=0.41$, $p=0.53$).

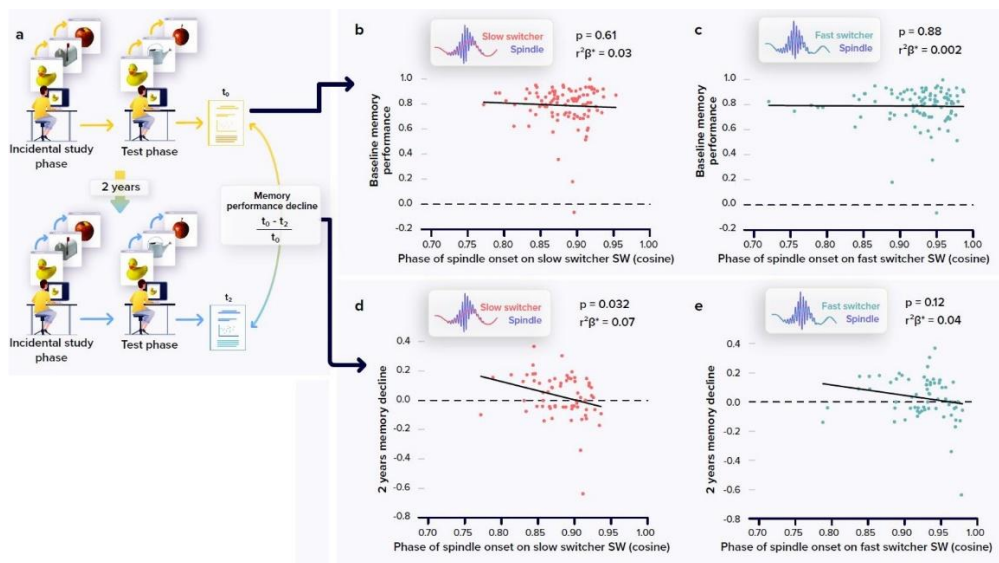


Figure 3. Relationships between memory performance and coupling between spindles and SWs. Memory performance was assessed through the Mnemonic Similarity Task (MST) where participants have to recognized previously encoded images in series of new or lure images (see methods) (panel A). No association between the baseline MST performance and spindle-slow switcher SW coupling (panel B). No association between the baseline MST performance and spindle-fast switcher SW coupling (panel C). Significant negative association between the 2 years relative change in MST performance and spindle-slow switcher SW coupling (panel D). No association between the 2 years relative changes in MST performance and spindle-fast switcher SW coupling (panel D). *P* values and $r^2\beta^*$ were computed from GLMMs referred to in the text. Simple regressions were used only for a visual display and do not substitute the GLMM outputs. We used the cosine value of the phase of coupling in the GLMMs (see methods).

DISCUSSION

In order to unravel early associations between the microstructure of sleep and the burden of A β in the brain, and their cognitive implications, we collected polysomnography, PET and behavioral data in a relatively large sample of individuals without cognitive impairments or sleep disorders. To this end, we recruited individuals in late middle age (50-70y) that could only present slight age-related alterations in sleep and a very limited accumulation of A β protein in the brain for most subjects (Grothe et al., 2017). We investigated whether the coupling of spindles onto SWs, showing a slower and a faster frequency of transition from the down to the up states (slow and fast switcher SW) was associated to the

accumulation of A β over the medial prefrontal cortex. We further probed whether the coupling of spindles onto SWs was associated with the performance to a sensitive memory test, assessed at the time of the sleep and PET recordings and, longitudinally, 2 years later. The coupling of spindles onto the slow, but not the fast, switcher SWs was significantly associated with the A β PET signal assessed over the MPFC. Moreover, this coupling between spindles and slow switcher SW was significantly linked to the memory performance change detected 2 years after the initial assessment. Overall, our results provide compelling evidence that the link between sleep and the accumulation of A β over the MPFC, an early AD-related brain features, involves the precise and timely coupling of two key elements of NREM sleep, spindles and SW, and that this coupling bears a predictive value for the subsequent decline in memory performance. Our study does not indicate, at least not in these healthy and relatively young older adults, that the amount of spindles or SW generated overnight is associated with the accumulation of A β over the MPFC.

Sleep SWs provide a readout of the homeostatic sleep pressure and are more prevalent at the beginning relative to the end of the night (Achermann et al., 1993). In addition, both the density of spindles and SWs have separately been related to overnight consolidation of memory (Fernandez & Lüthi, 2020; Miyamoto et al., 2017). They actively take part in information transfer from hippocampic to neocortical networks and in synaptic plasticity (Miyamoto et al., 2017; Ulrich, 2016). Recent research has put forward the importance of their precise phase coupling during sleep, and reported an age-related difference in that coupling (Helfrich et al., 2018). In the younger individuals, spindles tend to reach their maximum around the cortical up-state of the SW, whereas in older individuals, spindles occur earlier on the depolarisation phase of the SWs. This earlier coupling between spindles and SWs is related to a poorer overnight memory retention (Helfrich et al., 2018), suggesting a sub-optimal neuronal interplay for the exchange of information during sleep in older individual. In line with a presumed suboptimal coupling between spindles and SW, we find that, when controlling for age, individuals for which the spindles occur earlier during the transition phase of the slow switcher SW, show higher A β over the MPFC and a worse memory change over time. We did not find any significant effect of the age of participants on the phase of the coupling between spindles and SWs. This is likely due in part to the restricted age range of our participants, but also to the variability existing between the individuals in the changes they undergo in their sleep during ageing. Importantly, the relationship we observed between the phase of the

spindle onset onto the SW and the MPFC A β burden shows the same directionality (i.e. earlier spindles onto SW) as the changes previously reported in older individuals.

SWs were previously characterised according to the frequency of their transition from the down- to the up-state, which reflects the relative synchronisation of the depolarisation of the neurons when generating a SW (Bouchard et al., 2021). Beyond the well-known decrease in the production of SWs in aging, the slow switcher SWs were relatively preserved in the older individuals compared to the fast switcher SWs. This finding suggests that the two populations of SWs constitute distinct elements of sleep microstructure. Three results confirm that the two types of SW –slow and fast switchers – behave differentially. First, sleep spindles show a preferential coupling only with the transition period from down-to-up state of the slow switcher SW, while spindles occurring concomitantly to fast switchers SWs do not occur at a specific phase of the SW. Furthermore, only the coupling of spindles onto slow switcher SWs was significantly associated to the early accumulation of A β in the brain. Finally, only the coupling of spindles and slow switcher SWs was predictive of the memory change after 2 years. Our results support that slow switcher SWs, the type previously reported to be relatively spared during aging (Bouchard et al., 2021), is important to the development of AD-related pathological changes, at least in the form of A β protein accumulation, and for the subsequent development of subtle alteration in the cognitive abilities, leastways over the memory domain.

Sleep spindles are considered as thalamic events. They are generated through the interplay between the inhibitory reticular nucleus and the excitatory thalamocortical neurons, which project to the cortical neurons that feedback in turns to the thalamus (Ulrich, 2016). In contrast, SWs are intrinsically cortical. They consist in the spontaneous alternations between down (hyperpolarized) and up (depolarized) neuronal states (Adamantidis et al., 2019). The SWs undergo, however, an influence from the thalamus reticular nucleus that contributes to the synchronization of distant neuronal populations (Adamantidis et al., 2019; Lee et al., 2004). Hence, although the exact neurophysiological origin of the functional associations between spindles and SWs remain to be established, the thalamus reticular neurons could arguably be involved. Our results would therefore indicate that the early aggregation of A β protein in the medial prefrontal cortex could disturb the thalamocortical interplay driving the coalescence of spindles and SWs. This chronic disturbance would then trigger the cognitive changes detectable after 2 years. The cross-sectional nature of our imaging data does not, however, allow to make inferences regarding the directionality of the relationship between the spindle-slow switcher SW coupling and the burden of A β protein. Evidence accumulates to show that

if AD-neuropathological hallmarks can affect the quality of sleep, sleep can also impinge onto these hallmarks (Ju, Lucey, & Holtzman, 2015; Van Egroo, Narbutas, Chylinski, Villar González, Maquet, et al., 2019b). Specifically relevant in the context of this study, the occurrence of the SWs has been associated with a transient increase in the glymphatic flow related to local variations in neuromodulator concentrations (Xie et al., 2013). It is therefore possible that the changes in the density of SW occurring during ageing affect both their coupling with spindles and the early accumulation of A β protein, or conversely.

We found that the density of either spindles or SWs is not related to the early accumulation of the A β protein in the medial prefrontal cortex. This suggests a specific role for the coupling of both elements of sleep microstructure. Moreover and as previously reported in an intermediate analysis of a subsample of the same study (Van Egroo, Narbutas, Chylinski, Villar González, Ghaemmaghami, et al., 2019), we do not find significant associations between more macroscopic measures, which are typically used to characterize sleep, and the accumulation of A β protein. The cumulated power of the oscillations generated in the delta band (i.e. the slow wave energy - SWE), and the proportion of slower oscillations over the delta power were not associated with PET measures. This finding argues against the idea that these rougher measures of sleep disruption constitute the earliest manifestations of the association between sleep and AD-related processes and contrasts with previous reports in a smaller sample of individuals older (> 70y) than our sample. Altogether, these discrepancies reinforce the idea that the alteration in the microstructure of sleep, consisting in the coupling of the spindles onto a specific subpopulation of SWs, as reported here, but also in the occurrence of micro-arousals during sleep we previously reported based on the same sample (Chylinski et al., 2021), shows a prior association with AD-related processes compared with the amount of slow brain oscillations generated during overnight sleep that may only be significantly associated at a later age, when the pathophysiological changes are already more substantial. In addition, the coupling of spindles onto slow switcher SWs is predictive of the future change in memory performance. Sleep microstructure could therefore constitute a promising early marker of future cognitive and brain ageing trajectory (Chylinski et al., 2021). We did not evaluate whether distinct links between the SW types and slow and fast spindles are observed. As some reports describe that fast spindles are rather coupled to the up-state of the SWs and slow spindles tend to occur on the waning depolarisation phase of the SWs (Mölle et al., 2011), we could hypothesise that the associations we observe are probably rather driven by fast spindles. Future investigations are, however, needed to confirm this hypothesis

One should bear in mind the potential limitations of our study. First, although we collected data in a relatively large sample, we may have insufficient power to detect some associations with other sleep measures. One can nevertheless frame our findings in relative terms such that association between this coupling and the early accumulation of A β protein is at least stronger than the association with the coupling of spindles onto fast switchers SW, the density of SWs and spindles, the SWE, etc. Also, the longitudinal aspect of our study is relatively short-termed and only concerned the performance to a sensitive mnemonic task while it did not include sleep EEG and the PET assessments. Further studies should evaluate the predictive value of such parameters on longer longitudinal protocols, and the evolution of the sleep EEG and the PET parameters as well as their generalisability over other precociously impacted cognitive abilities. Given that our protocol does not include manipulation of the coupling of the spindles onto the SWs, it precludes any inference on the causality of one aspect onto the other.

Together, our findings reveal that the timely occurrence of spindles onto a specific type of SWs showing a relative preservation in ageing seems to play a determining role in ageing trajectory, both at the cognitive level and with regards to structural brain integrity. These findings may help to unravel early links between sleep, AD-related pathophysiology and cognitive trajectories in ageing and warrants future clinical trials attempting at manipulating sleep microstructure or A β protein accumulation.

METHODS

Study design and participants

101 healthy participants aged from 50 to 70 y (68 women; mean $\pm$ SD = 59.4 $\pm$ 5.3 y) were enrolled between 15 June 2016 and 2 October 2019 for a multi-modal cross-sectional study taking place at the GIGA-Cyclotron Research Centre/In Vivo Imaging of the University of Liège (Cognitive fitness in aging – COFITAGE – study) which has already led to several scientific publications [e.g. (Chylinski et al., 2021; Rizzolo et al., 2021)]. The exclusion criteria were as follows: clinical symptoms of cognitive impairment (Mattis Dementia Rating Scale >130 ; Mini-Mental State Evaluation (MMSE) >27) ; recent history psychiatric history or severe brain trauma ; self-reported or clinically diagnosed sleep disorder ; Body Mass Index (BMI) ≤ 18 and ≥ 29 ; use of medication affecting sleep or the central nervous system; smoking; excessive alcohol (>14 units/week) or caffeine (>5 cups/day) consumption; shift work in the

6 months or transmeridian travel in the 2 months preceding the study. All participants gave their written informed consent prior to their participation. The study was registered with EudraCT 2016-001436-35. All procedures were approved by the Hospital-Faculty Ethics Committee of ULiège. All participants signed an informed consent prior to participating in the study.

Table 1: Sample characteristics

Sex	68 ♀ / 33 ♂
Age (years)	59.44 ± 5.29 [50-69]
Education (years)	15.25 ± 3.01 [9 - 25]
Total sleep time (TST) (minutes, EEG)	393.23 ± 46.13 [229 - 495.5]
Time spent in N1 sleep stage (% of TST, EEG)	6.21 ± 2.76 [0.60 - 15.61]
Time spent in N2 sleep stage (% of TST, EEG)	51.67 ± 8.83 [31.42 - 75.71]
Time spent in N3 sleep stage (% of TST, EEG)	19.14 ± 6.41 [7.17 - 38.28]
Time spent in REM sleep (% of TST, EEG)	22.98 ± 6.61 [6.50 - 35.39]
Mean SW density (number/minute of N2/N3)	7.07 ± 4.32 [0.77 - 19;17]
Slow switchers	4.94 ± 3.06 [0.59 - 15.93]
Fast switchers	2.13 ± 1.61 [0.08 - 8.80]
Mean SW amplitude (μV)	101.52 ± 12.43 [75.98 - 128.25]
Slow switchers	104.55 ± 13.65 [77.73 - 131.30]
Fast switchers	93.98 ± 11.04 [72.99 - 130.09]
Mean SW frequency	1.22 ± 0.11 [1.04 - 1.62]
Slow switchers	1.11 ± 0.06 [0.98 - 1.32]
Fast switchers	1.50 ± 0.11 [1.26 - 1.81]

Mean SW transition frequency	1.37 ± 0.14 [1.09 - 1.81]
Slow switchers	1.08 ± 0.04 [0.98 - 1.17]
Fast switchers	2.04 ± 0.08 [1.88 - 2.24]
Mean spindle density (number/minute of N2/N3)	8.26 ± 1.06 [5.69 – 10.84]

BMI, alcohol consumption, anxiety & depression scores as well as sleepiness levels of the sample can be found in (Chylinski et al., 2021)

Sleep assessment

A first night of sleep was recorded at the laboratory under full polysomnography to avoid potential first night effects and exclude volunteers with sleep apnoea (AHI ≥15/h). A second night of sleep was recorded with electroencephalography (EEG), following one week of regular sleep-wake schedule based on each participant's preferred bed and wake up time (compliance was verified by actimetry and sleep diary - Actiwatch®, Cambridge Neurotechnology, UK). Sleep was recorded with N7000 amplifiers (EMBLA, Natus, Planegg, Germany). The recording comprised 11 EEG derivations, placed according to the 10-20 system (F3, Fz, F4; C3, Cz, C4; P3, Pz, P4; O1, O2), 2 bipolar electrooculogram (EOGs), and 2 bipolar submental electromyogram (EMG) electrodes. Sampling was set at 200 Hz, and the signal was re-referenced to the mean of the two mastoids. Recordings were scored for sleep stages in 30s windows using a validated automatic algorithm (ASEEGA, Physip, Paris, France) (Berthomier et al., 2007; L Peter-Derex et al., 2020). Automatic arousal and artefact detection (Chylinski et al., 2020; Coppieters 't Wallant, Muto, et al., 2016) was performed in order to remove EEG segments containing artefacts and arousals from further analysis.

Slow waves and spindle detection

Only the frontal electrodes were considered because the frontal cortex is an early site showing Aβ deposit and is the primary generator of the SWs during sleep (Dang-Vu et al., 2005, 2010; Mander et al., 2015; Saletin et al., 2013) as well as to facilitate interpretations of future large-scale studies using headband EEG restricted to frontal electrodes (Lucey et al., 2019). SWs were automatically detected during N2 and N3 epochs of NREM sleep devoid of artefacts/arousals >5s long, using a previously developed algorithm (T Rosinvil et al., 2020). Data were first band-filtered between 0.3 and 4.0Hz with a linear phase Finite Impulse

Response (FIR) filter. Following recent work, SW detection criteria were adapted for age and sex (T Rosinvil et al., 2020): peak to peak amplitude $\geq 70\mu\text{V}$ (resp. $\geq 60.5\mu\text{V}$) and negative amplitude $\leq -37\mu\text{V}$ (resp. $\leq -32\mu\text{V}$) was used for women (resp. for men), instead of the standard $\geq 75\mu\text{V}$ and $\leq -40\mu\text{V}$). The duration of the negative deflection had to fit in the range 125-1500, and the duration of the positive deflection could not exceed 1000ms. The SWs were sorted according to their transition frequency (Bouchard et al., 2021) (inverse of the duration between the hyperpolarised and depolarised state) into either slow ($<1.2\text{Hz}$) or fast switchers ($\geq 1.2\text{Hz}$). Sleep spindles were also automatically detected over the same N2 and N3 epochs with a previously published method (Gaudreault et al., 2018; Lafortune et al., 2014; N. Martin et al., 2013). The EEG signal was bandpass filtered between 10 and 16Hz with a linear phase finite impulse response filter (-3dB at 10 and 16Hz). After detection of SW and spindles, analysis of their coincidence was performed. A coincidence was defined as a co-occurrence of both the ignition and the maximum amplitude of a spindle over the phase of a slow wave. This criterion was used on slow and fast switchers.

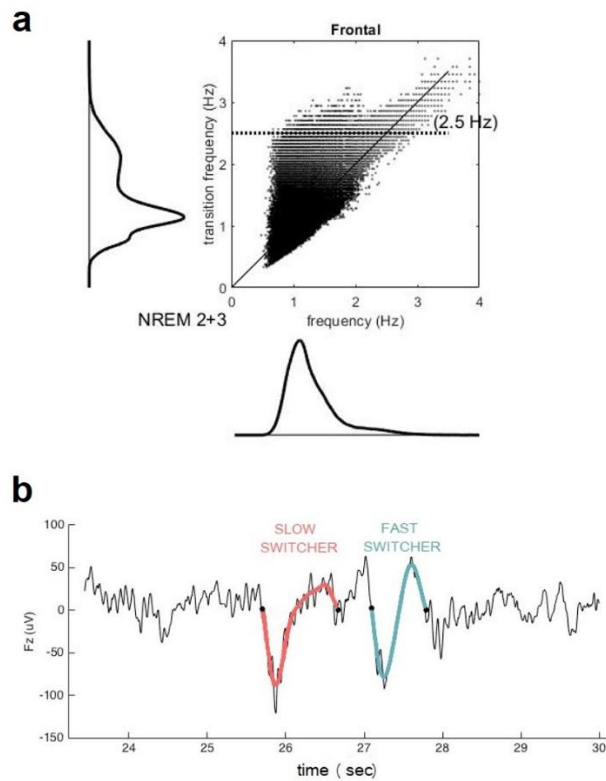


Figure 4. Distribution of the mean frequency of the slow waves (x axis) versus their transition frequency (y axis) for both NREM2 and NREM3 sleep stages (panel A) in the entire study sample.

One can observe a double distribution of the frequency of transition but not in the overall frequency. Example of slow switcher (red) and fast switcher SW (light blue) extracted from the EEG signal.

MRI data

Quantitative multi-parametric MRI acquisition was performed on a 3-Tesla MR scanner (Siemens MAGNETOM Prisma, Siemens Healthineers, Erlangen, Germany). Quantitative maps were obtained by combining the images using different parameters sensitive to distinct tissue properties. The multi-parameter mapping was based on multi-echo 3D fast low angle shot at 1 mm isotropic resolution (Weiskopf & Helms, 2008). This included three datasets with T1, proton density (PD), and magnetization transfer (MT)–weighted contrasts imposed by the choice of the flip angle ($FA = 6^\circ$ for PD & MT, 21° for T1) and the application of an additional off-resonance Gaussian-shaped RF pulse for the MT-weighted acquisition. MRI multi-parameter maps were processed with the hMRI toolbox (Tabelow et al., 2019) (<http://hmri.info>) and SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK) to obtain notably a quantitative MT, which was segmented into grey matter, white matter, and CSF using unified segmentation (Ashburner & Friston, 2005). Flow-field deformation parameters obtained from DARTEL spatial normalisation of the individual MT maps were applied to the averaged co-registered PET images (Ashburner, 2007). The volumes of interest were determined using the automated anatomical labelling (AAL) atlas (Tzourio-Mazoyer et al., 2002).

PET-scan

A β PET imaging was performed using [^{18}F]Flutemetamol, except for 3 volunteers for which [^{18}F]Florbetapir was used. PET-scans were performed on an ECAT EXACT+ HR scanner (Siemens, Erlangen, Germany). Participants received a single dose of the radioligand in the antecubital vein (target dose $185 \pm 10\%$ MBq); image acquisition started 85min after the injection and consisted of 4 frames of 5 minutes, followed by a 10 minutes transmission scan using ^{68}Ge line sources. Images were reconstructed using a filtered back-projection algorithm including corrections for the measured attenuation, dead time, random events, and scatter using standard software (Siemens ECAT – HR + V7.1, Siemens/CTI Knoxville, TN, USA). Individual PET average images were produced using all frames and were then manually reoriented according to MT-weighted structural MRI volumes and coregistered to the individual space structural MT map. Standardised uptake value ratio (SUVR) was computed

using the whole cerebellum as reference region (Klunk et al., 2015). As images were acquired using 2 different radioligands, their SUVR values were converted into Centiloid Units (Klunk et al., 2015) (the validation of the procedure in our sample was previously published (Narbutas, Van Egroo, et al., 2021)). The A β burden was averaged over a mask covering the medial prefrontal cortex previously reported to undergo the earliest aggregation sites for A β pathology (Grothe et al., 2017).

Cognitive assessments

As part of an extensive neuropsychological assessment, participants were administered the Mnemonic Similarity Task (MST) (Stark, Stevenson, Wu, Rutledge, & Stark, 2015), a visual recognition memory task. After an incidental encoding phase during which participants were randomly presented 128 common objects for a period of 2s, and were instructed to determine whether the object presented on the screen was rather an ‘indoor’ or ‘outdoor’ item, the recognition memory phase consisted in the presentation of 192 objects (64 old, presented previously – target items; 64 similar but not identical to the previously presented stimuli – lure; and 64 new objects – foil items). In this phase, participants were instructed to determine whether the presented object was new (foil), previously presented (old), or similar but not perfectly identical (lure). For statistical analyses, the recognition memory score was used (RM), computed as the difference between the rate of calling a target item “old” minus the rate of calling a foil item “old” [$P(\text{“old”}|\text{target}) - P(\text{“old”}|\text{foil})$] (Rizzolo et al., 2021; Stark et al., 2013).

The MST was administered at two timepoints: the first time, the day preceding the baseline night, during a cognitive evaluation performed ~ 6.5h before habitual bedtime. The second neuropsychological evaluation was carried out ~24 months after the first one. The memory decline score was computed as the baseline performance minus the follow-up performance, divided by the baseline performance, so that a higher score indicates a higher decline over the 2 years.

$$\text{memory decline} = \frac{\text{RM baseline} - \text{RM follow-up}}{\text{RM baseline}}$$

Statistics

Statistical analyses were performed using Generalised Linear Mixed Models (GLMMs) in SAS 9.4 (SAS Institute, Cary, NC). The distribution of dependent variables was

verified in MATLAB 2013a and the GLMMs were adapted accordingly. Subject was treated as a random factor and each model was corrected for age and sex effects. Statistical significance was set at $p < 0.05$. Kenward-Roger's correction was used to determine the degrees of freedom. 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 et al., 2017b). P-values in post-hoc contrasts (difference of least square means) were adjusted for multiple testing using Tukey's procedure. Watson's non-parametric two-sample U^2 test for circular-normal data was performed in MATLAB 2019 to assess the difference between the distribution of spindle onset on the phase of slow waves for slow and fast switcher SW. For analyses using the phase of spindle onset on the slow waves, as the phase of all subjects were in the quarter between the zero crossing ($-\pi/2$) and depolarisation, the cosine of the phase was used instead of the phase, in order to perform linear statistics. Statistics with the phase yielded the same results. We used the Causalmed procedure in SAS to estimate causal mediation effects. The outcome variable was the memory decline, the treatment variable was the coupling of the spindles onto slow switcher SWs, the mediator was the A β burden in the MPFC and the background covariates were age, sex and education. An interaction effect between the treatment and the mediator variables was included in the model. We report the controlled direct effect, as the component effect that is not due to interaction or mediation, and the pure indirect effect, as component effect that is due to mediation and not interaction.

General discussion

Preamble

It has been established that pathological processes in the form of progressive accumulation of beta-amyloid plaques and tau neurofibrillary tangles start taking place many years before any clinical sign of cognitive decline can be detected (Duyckaerts & Hauw, 1997). Current techniques, such as PET scans and lumbar punctions, help estimate the level of amyloid and tau brain deposition in vivo. However, these biomarkers just indicate an increased risk of developing AD, and do not help predict whether and when cognitive symptoms will appear. Moreover, it is important to note that there is currently no efficient treatment for AD, especially when cognitive decline becomes manifest. Current clinical trials attempt to reduce A β and tau burden during the preclinical stage in individuals at higher risk of developing AD, because of mutation in APOE gene or because of an already high load in protein. Importantly, preventive measures, prior to marked cognitive decline, that are focused on features not typically considered as part of AD neuropathology are promising to decrease AD incidence (e.g., prevention of vascular diseases, adapted diets and physical exercise). Yet these strategies will only partly reduce the burden of AD. It is therefore crucial to identify new risk factors and biomarkers for AD to rethink prevention strategies. This would improve risk prediction prior to clinical intervention and will help if not preventing the disease, at least delay it substantially which would already be considered as a major achievement. The present PhD thesis works arises from this will to enlarge AD perspective by focussing on sleep as a potential indicator of risk and modifiable factor that may help delay disease onset.

Sleep is a physiological state which is central to both health and cognition (Deak & Stickgold, 2010; Luyster, Strollo, Zee, & Walsh, 2012). Its modifications across the lifespan have been well documented (Ohayon et al., 2004), and alterations are common in the process of ageing (Lavoie, Zeidler, & Martin, 2018). Beyond healthy ageing, deteriorations in sleep are associated with the risk of developing AD over the following years (Lim et al., 2013; Musiek & Holtzman, 2016). Sleep features have been associated to A β and misfolded tau protein accumulation, as well as neurodegeneration, the three main hallmarks of AD (Van Egroo, Narbutas, Chylinski, Villar González, Maquet, et al., 2019a).

Brain dynamics supporting cerebral function represent constant interactions between neuronal populations. Sleep stages can be viewed as representing larger time windows composed of a widespread oscillatory mode. Sleep stages are however a simplistic outline of the inherently labile fluctuations of neuronal patterns, serving its purpose of summarising the

global dynamics of sleep. When looking at the EEG in a smaller time frame, one can appreciate the diversity of the ongoing oscillations. It is these oscillations that are arguably fulfilling the multiple functions of sleep. Brain burden of aggregated A β and misfolded tau have been linked to a reduced generation of slow waves during sleep and the coupling between slow waves and spindles, while A β burden was reportedly elevated in OSA patients (Elias et al., 2018; Mander et al., 2015; Winer et al., 2019). Conversely, experimental disruption of slow wave sleep has been shown to lead to higher concentration of A β in the CSF (Ju et al., 2017). Together, these results imply a bidirectional relationship between sleep and AD biomarkers (Ju, Lucey, & Holtzman, 2015). The aim of the present dissertation was thus to investigate the early links between alterations in sleep microstructural characteristics and biomarkers of AD neuropathology, and cognitive trajectory in otherwise healthy older individuals.

Methods and their limitations

We first started the experimental part of this dissertation with two chapters addressing methodological issues related to sleep scoring. Our first empirical goal was to investigate arousal links with AD-related neuropathological processes. In the first method article, we therefore aimed to validate a method for the automatic detection of arousals events in the sleep EEG that had been previously developed for the undifferentiated detection of both artefacts and arousals (D Coppieters 't Wallant et al., 2016). The detection of sleep microstructural elements is subject to intra- and inter-rater variability, which needs to be taken into account when comparing results across studies. Although the overall inter-rater agreement was very good, as reflected by agreement's coefficient S as well as Kappa's κ values, the results showed a relative difference in arousal counts between visual scorers, as well as the difference in arousals detected automatically. Automatic detection methods are known to yield a higher count of detected events than visual scoring.

The other methodological aspect, addressed in the second chapter of the experimental protocol part of this work, is related to the variability of scoring sleep stages in older individuals. All the empirical papers of this thesis are indeed based on COFITAGE sample of late midlife individuals. Given the known sleep alterations present in the ageing process, we wished to specifically confront sleep stage scoring of independent scorers in the context of an older population. Surprisingly, few papers directly addressed scoring variability in ageing including using an automatic algorithm. We found a higher inter-scorer variability than what is usually reported in the literature, particularly for the distinction between N2 and N3 stages,

while agreement was high for REM sleep. Crucially, the scoring criteria allowing to distinguish between those sleep stages are related to the appearance of slow waves with a specific amplitude criteria of 75 μ V. A previous study showed that beyond the age-related difference in the ability to generate slow waves, a general drop in the amplitude of slower oscillations is seen, while adapting slow wave detection threshold for age (as well as sex) allows for a more comprehensive inventory of the generated slow waves, without abolishing the age-related effect (T Rosinvil et al., 2021). Those results stand in favour of a re-evaluation of the amplitude criteria for the detection of slow waves or the scoring of N2/N3 sleep stages in older populations, or, alternatively, to regard N2 and N3 sleep stages as a whole. The use of automatic scoring algorithms, such as ASEEGA (Berthomier et al., 2007), which do not rely on absolute amplitude criteria, but rather on data-driven individual thresholds, also constitutes an interesting way around this issue.

To conclude these methodological considerations regarding the scoring of sleep, we would like to underline, in our era of big data studies, the increasing need of replicability and comparability. Although human visual scoring remains the gold standard, we emphasise that both visual and automated methods rely on the interpretation of scoring criteria and rules, which, in the case of visual scoring, is exposed to subjective interpretation of the scorer and can be influenced by several factors such as fatigue of the rater, while for automatic scoring, they require an implementation of scoring rules and sometimes the choice of arbitrary thresholds. Though both methods have supporters and challengers, it is becoming clear now that, while automatic algorithms show similar agreement with visual scoring than visual scorers between themselves, they have the advantage of providing a fast and replicable alternative to the tedious task of visually scoring sleep.

Study I – Sleep arousals are heterogeneous

The first chapter of the results section focused on sleep arousals, which are transient changes in oscillatory activity involving faster frequencies. The main result from this chapter is the heterogeneity in spontaneous sleep arousals we find, which is reflected in their different oscillatory composition. We differentiated arousals based on two aspects which we deemed clinically relevant: the co-occurrence of an increase in muscular tone, or its absence (M+/-), and whether the arousal lead to a stage transition or not (T+/-). We found that T+M- arousals were associated with a higher cortical A β burden over earliest affected regions, while more T-

M+ arousals were related to a lower cortical A β burden. Moreover, the latter type of arousals was associated to a better cognitive performance, particularly over the attentional domain.

One very interesting aspect would be to corroborate our results regarding the heterogeneity of arousals by confronting our criteria to clinical events, such as respiratory activity or limb movements. This data was not available on the baseline nights of the protocol, though cardiac activity is available for a subsample of our study. Nasal flow and belt –thoracic and abdominal- signals are available from the habituation nights recording, which are not an ideal source of data as they were not performed under condition of controlled circadian and homeostatic pressure and can be impacted by the so-called first night effect, as sleeping in a novel environment has been shown to impact sleep metrics (Agnew et al., 1966). Nevertheless, analysing the screening night in our sample could constitute an exploratory first indication of whether events related to sleep pathologies are related to a specific type of arousals, even though our population was screened for those and would therefore exhibit a very limited number of such events. We could also assess how arousal are related to A β when they are separated into sleep-pathology related arousal and spontaneous arousals. Alternatively, a collaboration with the sleep lab at the CHU of Liège, where our automatic arousal detection algorithm could be used to investigate the recording of a clinical population, would constitute a different way of validating our arousal distinction criteria, though it would not be possible to corroborate it with brain imaging data.

Interestingly, one additional analysis not presented in the final version of this paper concerned the analysis of the potential heterogeneity of arousals using a data-driven exploratory approach. We performed a principal component analysis (PCA) on the spectral composition of all arousal events from our whole dataset, which resulted in two main components responsible for most of the variance. Once the data was transformed into this two-dimensional plane, a silhouette plot revealed two clusters in our dataset (see appendix 3). The output of this analysis, though not related to the two dimensions reported in the main analysis in the paper, reinforces us in the idea of an inherent heterogeneity in spontaneous sleep arousals, which could have different functional consequences.

Additionally, one aspect we didn't explore in this work was the dynamical aspect of arousals in relation to the cyclic alternation between sleep cycles. Indeed, as it was previously shown that the ascending slope of sleep cycles is not the mere reflection of the descending one, as it is usually ~50% shorter and presents more stage 'jumps', where some stages are skipped

in the depth-order of sleep stages (Halasz & Bodizs, 2012). It is therefore possible that arousals, especially the ones leading to a stage transition, may have a differential impact depending on whether they occur on the descending or ascending slope of a given cycle. One report described such a difference between arousals in NREM according to the slope of the cycle, and mentions that arousals on the descending slope tend to be longer, and would carry a function of sleep promotion, unlike arousals on the ascending slope of cycles, which would be more desynchronised, with autonomic signs, and are assumed to prepare REM sleep (Halasz et al., 2004). It would therefore be of great interest to confront those results to our dataset and compare the differentiation of arousal types. Likewise, to the best of our knowledge, the circadian aspect of arousals has never been investigated, and analysing their occurrence with regards to whether they mostly occur at the beginning or towards the end of the sleep episode would allow us to understand the influence they have from the circadian system. Finally, given the obvious differences in the oscillatory regime in NREM and REM sleep, one could hypothesise that arousals, which represent a shift towards higher frequency oscillations, have potentially different meaning depending on whether they occur in NREM or REM sleep, as both those sleep stages are characterised by different sensory reactivity. Nevertheless, data from our analysis tend to suggest that the relationships we observed between arousal types and A β burden are not related to a particular sleep stage.

Interestingly, the neurophysiological pathways underlying arousals remain elusive. Orexin, a neuropeptide involved in the initiation and maintenance of wakefulness, has been suggested to modulate A β pathology through its effect on sleep and wakefulness regulation (Roh et al., 2014). It seems plausible to postulate that orexin could play a role in the generation of some arousals, possibly those leading to a transition towards a lighter sleep stage, and thus contribute to the accumulation of A β , or vice versa, though animal models would need to corroborate their implication in arousal generation. Additionally, the recording of sleep via combined EEG/fMRI could provide insight into the differentiation of arousal types, with all the limitations that this technique imply.

Study II: Slow waves are associated with neurodegeneration

In the second chapter, we investigated the brain tissue microstructure associated with the ability or the need to generate slow waves during NREM sleep, using multi-shell diffusion weighted imaging and NODDI post-processing. Fundamentally, slow waves consist in the synchronised alternation between active periods (“up” state) and silent periods (“down” state)

among neuronal populations (Vladyslav V. Vyazovskiy, Riedner, Cirelli, & Tononi, 2007), reflected at the EEG level by slow frequency oscillations. As previously reported, a differentiation in slow wave oscillations present in the literature (P. Achermann & Borbély, 1997; Lee et al., 2004) was recently proposed based on the transition frequency of slow waves, that is, the transition from down- to up-state, reflecting synchronised depolarisation (Bouchard et al., 2021). Our results seem to imply that neurite coherence within regions underlying slow waves, impact slow waves density. A higher branching density of the dendritic arborisation, reflecting a greater grey matter complexity, may help maintain the generation of slow waves, potentially by facilitating neural synchronisation, particularly for slow waves with a higher transition frequency, previously shown to be reduced in ageing (Bouchard et al., 2021). The other main result of this chapter is the positive association between the mean frequency of slow waves and the density of neurites, suggesting its role for the synchronous reaching of active and silent states by neuronal populations.

Other cross-sectional studies already reported a relationship between grey matter integrity measures, such as cortical volume or thickness and sleep features (A.-A. Baril et al., 2021; J. Dubé et al., 2015; Van Someren et al., 2018). NODDI has the advantage of offering a greater insight into cerebral tissue microstructure. A previous study reported that age-related modifications in grey matter structure are better captured by multi-compartment models such as NODDI compared to single-tensor diffusion (Venkatesh, Stark, Stark, & Bennett, 2020), and was shown to be a reliable tool to assess tissue microstructure *in vivo* (K. Sato et al., 2017). Importantly, a recent study showed that individuals with MCI had lower NDI in temporal and parietal cortices, while cortical thickness was not affected, suggesting that NDI might be a very sensitive marker for the fine gradient changes occurring prior to measurable cortical atrophy (Vogt et al., 2020). While research using this relatively new, state-of-the-art imaging method is still in its infancy, it might constitute a promising tool for the assessment of early changes in brain integrity taking place in ageing, in human as well as in animal models. NDI has been previously related to increased tau burden in mice (Colgan et al., 2016). This is of particular interest since it is conceivable that SW would be a modulator of microstructural alterations and pathophysiological processes related to AD (i.e. the accumulation of A β and tau).

While in this chapter, we were specifically interested in the relationship between brain microstructural aspects in a region crucial for the generation of SW and sleep SW characteristics, NODDI could be of interest in the investigation of age-related cerebral changes

in relation to cognition. Indeed, a study using NODDI in white matter (WM) on healthy controls, MCI and AD patients showed that NDI was the metric which showed highest sensitivity to group differences, while both ODI and NDI were associated with cognitive score (Fu et al., 2019). Lower WM NDI in frontal regions was also shown to correlate with poorer cognitive performance (Merluzzi et al., 2016).

Study III: The fine-tuned coupling between sleep events is related to early A β

In the third chapter of our results, we present data showing that the precise co-occurrence of sleep microstructural elements may be as relevant for cognitive and cerebral integrity as their independent manifestation. We examined the timely coupling between spindles and slow waves and found that spindles exhibit a preferential coupling phase only on slow switcher slow waves, which have a lower transition frequency. This result in itself is of importance, as it tends to support of a general distinction in the slower oscillations during NREM sleep, and imply a different functional role for both slow wave types.

We also found that this coupling, previously reported as impacted in ageing, and involved in overnight memory processing (Helfrich et al., 2018) was associated with A β burden over the medial prefrontal cortex, and was predictive of the memory performance change over the 2 years follow up, measured by the Mnemonic Similarity Task, a visual memory task sensitive to early memory decline (Rizzolo et al., 2021; Stark et al., 2013). It seems particularly interesting and important that, despite the relatively young age of our participants as well as the short duration of the longitudinally assessed cognitive data, we still observed a significant relationship between spindle and slow wave coupling and cognitive change at the 2 years follow-up.

Recent work by Winer and colleagues investigated the coupling between slow waves and spindles, and showed it was related to cortical tau burden over the medial temporal lobe (Winer et al., 2019). In that study, the authors focused on the coupling strength, and not on the phase of the spindle onset on slow waves. In analysis not reported in the paper, we also investigated coupling strength and its relation to A β burden, and failed to find such a relationship.

Importantly, while the transition frequency has recently been proposed as a way of distinguishing between slow sleep oscillations, it remains unaddressed how slow and fast switchers relate to slow oscillations (<1Hz) and delta waves (1-4Hz). Interestingly, plotting

slow waves according to their main and transition frequency (see figure 12 in the second chapter of the introduction, and figure 4 in chapter VIII) shows a mixture of gaussians only for the transition frequency, and not for the main frequency of slow waves. The relationship between slow and fast switcher SW and slow oscillations and delta waves does not seem straightforward, and would require further research to be elucidated.

How sleep may be related to AD

Interestingly, further exploratory analysis showed a significant negative relationship between the memory change at 2 years and the T-M+ arousal index, further reinforcing the idea of their positive role (see appendix 4). It seems thus that arousals are an important aspect of the sleep EEG, while remains unclear for the moment why they are associated with attentional function at baseline and with memory functioning on the long run. It is of course possible that arousals are related to other microstructural aspects, such as SW or spindles. In fact, exploratory analysis presented in appendix revealed a positive correlation between the spindle coupling with slow switcher SW and T-M+ arousals, related to better cognitive performance. It seems therefore that a higher prevalence of those positive arousals is related to a later onset of the spindle on the phase of slow switcher SW. This constitutes an interesting element suggesting that both those aspects are related, though the mechanistic pathway remains elusive. Arousals were reported to be the resultant of a necessity to keep the sleeper's brain connected to the environment, while trying to conserve the state of sleep (Halasz et al., 2004). A stereo-encephalographic study reported the a stereotyped activity in the thalamus during arousals, with a spectral composition reflecting a state in-between wakefulness and sleep (Laure Peter-Derex, Magnin, & Bastuji, 2015). Given the implication of the thalamus both for slow waves and spindles generation/modulation (Adamantidis et al., 2019; Lee et al., 2004), as well as potentially in arousals (Zou et al., 2019), and the implication of the thalamus as a gateway to the brain (Ward, 2013), one could consider its importance for the ageing process. It would thus be very interesting to investigate the potential relationships between thalamic characteristics, and sleep microstructure.

A main publication linking A β accumulation to a cruder measure of sleep microstructure is the work by Mander and colleagues (Mander et al., 2015). They showed that the A β burden assessed over the MPFC is correlated to the level of SWA in the 0.6-1Hz range, as well as with the proportion of SWA over the spectral power between 0.6-4Hz. Moreover, they showed that the reduction in SWA was associated with impaired overnight memory

retention. This paper constitutes an important part of the literature linking sleep, brain integrity and cognition. However, the study based only on a limited sample of older individuals (26 subjects, aged $\pm$ 75 years), and to our knowledge, failed to be replicated so far. In our sample of 100 individuals, we did not find a relationship between the cumulated power in the delta band or slow oscillations and MPFC A β burden. Analysis of the density of slow and fast switchers, another way of distinguishing oscillations in the 0.5-4Hz frequency range, yielded similar results. We have to concede, however, that our sample was constituted of younger individuals, aged 50-70y. It is possible that this relationship is only observable in older individuals, where higher A β deposits are observed. One possible interpretation of these discrepancies resides in the possibility that, before affecting the generators of slow waves, A β burden first affects the fine-tuned coalescence of sleep rhythms, as reflected by the co-occurrence of slow waves and spindles, and arousals. The cross-sectional nature of our data does not allow us, however, to make inferences on the directionality of the relationships we observed, which is likely to go both ways. Given all these results, it seems plausible to hypothesise that sleep microstructure represents the earliest impacted aspect of sleep and would precede changes observable on less refined characteristics of sleep, such as the amount/amplitude of produced slow waves, the percentage of total sleep time spent in slow-wave sleep, and sleep continuity.

While the central role of this dissertation was to further our understanding of the core associations between sleep and pathophysiological processes related to AD, the far-reaching aim of this whole part of literature is to identify novel markers that could be relevant for the early diagnosis and prognosis of adults which could be considered at higher risk of developing AD. As such, it seems the road leading to the clinical implementation of our findings seems to remain relatively long. Nevertheless, provided some work regarding the improvement and implementation of automated detection tools in clinical settings, one could imagine it could constitute one of the tools used to assess sleep in the context of AD, for its prevention, early detection, at the same level as actimetry (Jia Li et al., 2018), and potentially even treatment, for instance through SW enhancement (D. J. Dijk, 2010; Wilckens, Ferrarelli, Walker, & Buysse, 2018).

Is sleep so important for AD?

We have to acknowledge that the size of the effects reported in the results section of the present dissertation remain modest. It has been argued, however, that small effect sizes,

though not central to the explanation of variance of single events, can have great importance on the longer run (Funder & Ozer, 2019). Detrimental microstructural aspects, if repeated on the long term, can therefore give rise to non-negligible negative consequences.

In the present work, we investigated the relationships between sleep and increased risk factors for the development of AD, in the form of two of its hallmarks – A β accumulation, in the first and second chapters, as well as neurodegeneration in the third one, as well as its relation to cognitive performance. But what about sleep's counterpart – wakefulness? Is it that those alterations observed at the level of the EEG are limited to the sleeping brain and cannot be observed during wakefulness? Or perhaps those sensitive and discrete changes are masked in the awaked state, because of the many factors that may impede the investigation of wake EEG? The data necessary to answer these questions is in our hands, as the COFITAGE protocol involved multiple resting state EEG recordings, performed at several time points across the wake extension protocol. Some insights have been provided by the thesis of my colleague, Dr Van Egroo, where he investigated the wake-dependent regulation of cortical function. Let us hope someone takes up the torch to explore those aspects and help us elucidate this topic.

It is plausible that the limited size of the effects we observed are due to features other than sleep, which might contribute to the investigated relationships, possibly in a greater way. It is central to remember that sleep is not the only relevant aspect related to AD pathophysiology. Indeed, other pertinent facets not addressed in this thesis have also been linked to the risk of future development of AD. Cognitive performance has been shown to be under the combined influence of a variety of factors, some protective, other adverse, including genetic, environmental and sociodemographic factors which have all been investigated in the thesis of my colleague, Dr Narbutas. It is therefore important to remember that humans are multidimensional beings, and to take all these characteristics into account when predicting ageing trajectories. AD is thus multifactorial, which is what renders it so difficult to prevent. Future research should consequently examine if and how sleep behave according to genetic, as well as environmental risk factors.

Perspectives – the unknowns that remain to be addressed

Though the results emerging from our three chapters allowed us to answer some questions, plenty other questions remain to be addressed. An interesting step to further our understanding of the relationship between sleep and structural brain changes would involve

the investigation of other sleep microstructural elements, such as vertex sharp waves, cyclic alternating patterns (CAP), or REM graphoelements such as REM-alpha bursts. Those elements remain under-investigated, especially with regards to ageing. The analysis of CAP, constituting a marker of sleep instability, would provide an interesting insight into the fluctuations of sleep dynamics, complementary to the analysis of arousals. Interestingly, several automatic CAP detectors have recently been developed (see (Qian et al., 2021) for a review), making the investigation of larger datasets more accessible.

Importantly, while in the third chapter, we focused on the memory decline, as assessed via the MST task, as it is one of the first aspects of cognition to exhibit age-related changes, the rest of the cognitive assessment at follow-up constitutes a wealth of information that remains to be investigated. While this follow-up assessment contains an overview of the main cognitive domains, it has to be noted that the time between the two neuropsychological evaluations remains short (2 years), particularly for such healthy individuals in late midlife, which, expectedly, will show only limited variation comparative to the baseline assessment. It would therefore be of great interest to continue the longitudinal assessment over an extended period of time.

Finally, another interesting analysis would be the inter-relationship between the different markers of brain structural integrity. A voxel-based analysis of quantitative parameters across imaging modalities – using data acquired via MRI as well as [^{18}F]Flutemetamol and [^{18}F]THK5351 PET assessments of A β and tau protein burden, respectively, is one of the further goals of the COFITAGE protocol, which will help gain insight into the relationship of those markers at the voxel level over the whole brain.

Overcoming the limits of COFITAGE

The choice of participants and their exclusion criteria are also criticisable aspects of this study. As for all studies, not only focusing on the impact of ageing, the selection bias represents a challenge to address. Many potential participants do not take part in the study, possibly in part because of the way individuals are recruited – via word of mouth, advertisements placed in the university, as well as during fairs e.g. organised by the city of Liège. In our sample, out of 101 participants, 26 had a secondary school diploma, 37 had a bachelor degree, and 38 had a master's degree or higher diploma, while in the overall Belgian population of that age range, ~33% have a lower secondary school diploma or lower, ~36% have a higher secondary school diploma, ~19% a bachelor degree and ~12% a master's degree

or higher², which means that individuals with lower education are clearly under-represented in our sample, as are males (33 subjects in our sample). Also, because of the demands of the protocol, which comprised no less than ~7 visits, one had to be relatively fit, and available to take part in our study. It is a well-known fact that individuals with a lower socio-economic status are a population that remains difficult to reach and make up for a small part of the studied samples in research (Henrich, Heine, & Norenzayan, 2010).

The restricted age range is most probably responsible for the lack of age effect that we observe in our data on features that have otherwise been reported to vary with age in the available literature. The very strict inclusion criteria for our protocol also constitute a debatable aspect of the study. The COFITAGE study enrolled individuals that were mostly devoid of many of the previously established risk factors associated with cognitive decline and AD, such as hypertension, midlife obesity, or smoking (Norton et al., 2014). This choice was motivated by a will to control for most factors that could influence the examined relationships between brain integrity, sleep, and cognition. Yet, considering that the individuals we recruited represent only a small portion of the population, the generalisability of our results to the general population seems compromised, and further replication of our results would need to be performed over a sample of all-comers. Nevertheless, the fact that we observed the described relationships in a sample characterised by relatively limited variability constitutes a very interesting outcome. It is possible that the microstructural elements we investigated would be of increased significance in the general population, and one could hypothesise that the links with AD pathophysiology would be further increased in a population of all-comers.

The cross-sectional nature of our sleep and brain imaging data is also an inherent limitation of the Cofitage study. It would of course be highly interesting to longitudinally evaluate the changes in sleep characteristics, which would enable us to evaluate if one sleep feature could predict another. For instance, if we consider that sleep microstructure is the fine gradient aspect of neuronal interplay taking place during sleep, it is possible that their alterations precede, and potentially predict impairments of other sleep characteristics. Besides, the cross-sectional nature of our data does not allow us to make inferences regarding the evolution of those features across the lifespan. Future longitudinal research comprising repeated measures of sleep EEG as well as PET imaging should establish and further elucidate

² <https://www.iweps.be/indicateur-statistique/niveau-de-diplome-de-population-de-25-ans-plus/>

if, and how, sleep predicts A β , as well as how both these measures might impact cognition on the longer run. It is indeed possible that the association we observed between the coupling of spindles and slow waves is due to the fact that this coupling is a forerunner of a worse sleep quality, and, alternatively or conjointly, of a higher A β burden at the time of the follow up.

What about tau?

One of the aspects we didn't investigate in the present work is the potential link between sleep and tau NFT. Though [^{18}F]THK5351 imaging data was included in the COFITAGE protocol, due to technical issues, it was only acquired for a subsample of the data (69 participants). Also, [^{18}F]THK5351 is a first-generation PET marker of tau NFT, which, besides its binding to tau NFT, was reported to show substantial off-binding towards MAO-B (up to 50%), associated with neuroinflammation, particularly over the basal ganglia (Chiotis et al., 2018; Lemoine et al., 2017). For those reasons, we decided not to privilege that marker in our analyses, though the data is available for future investigation which could examine the potential relationships it has with sleep characteristics while acknowledging those limits. Some publications from our group have already investigated that matter (Narbutas, Chylinski, et al., 2021; Van Egroo et al., 2021). The near future should see an increase in studies investigating the relationships between sleep and tau NFT burden, as third-generation radiotracers, showing little to no off-target binding will become increasingly available, allowing to reliably investigate the neurophysiological changes associated to tau protein (Bischof et al., 2021; Mittal et al., 2021). Given that sleep arousals, or at least some of them, most probably involve activation of brainstem areas such as the LC (Zou et al., 2019), involved in sleep and wakefulness regulation, and given the fact that the first intraneuronal lesions associated to tau aggregations are found in brainstem nuclei, particularly the LC (Heiko Braak et al., 2011), it seems reasonable to think that those two phenomena could be related.

Conclusion

In the exciting field of sleep research, almost one hundred years after the invention of the electroencephalogram by Hans Berger which helped gain new insights on the dynamics of sleep, we have to admit that a lot of the secrets around the patterns constitutive of the sleep EEG have not yet been unlocked. The present dissertation was part of the recent research effort to untangle the relationships between sleep microstructure, and cerebral and cognitive trajectory in the ageing process. Using a cross-modality approach, we showed that the microstructure of sleep, in the form of spontaneous sleep arousals, is associated with the level

of A β accumulation over the earliest deposition sites, and can be a marker of better cognition, in otherwise healthy individuals. We also showed that slow waves characteristics are associated with the neurite density and organisation, an aspect that can be associated with early neurodegeneration. Finally, we showed that the precise, timely occurrence of slow waves and spindles, previously shown to be related to overnight memory function, is also linked to A β burden over the medial prefrontal cortex, a crucial region for slow wave generation, and is predictive of a better memory change over a two years follow-up. Though some of our results are in line with previous findings and expand the insights those provided, other results open new questions, particularly regarding the role spontaneous arousals play in the sleeping brain.

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Appendices

Appendix 1. Supplemental data for chapter IV: Variability of sleep stage scoring in the elderly

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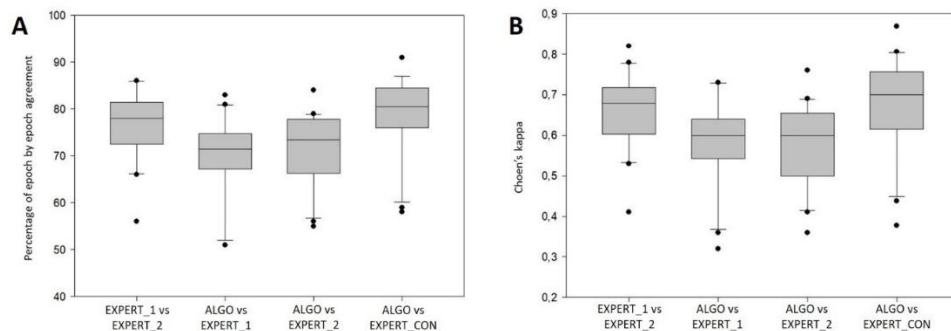
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Results on C4-O2



Supplementary figure 1. Boxplot of percentage of agreement (panel A) between EXPERT_1 and EXPERT_2, ALGO and EXPERT_1, ALGO and EXPERT_2 and ALGO and EXPERT_CON with

ALGO staging performed on C4O2 electrodes; boxplot of Cohen's kappa (panel B) for the same comparisons. The boxes' central line represents mean values while one dot corresponds to one recording, and outliers were not removed from the plot

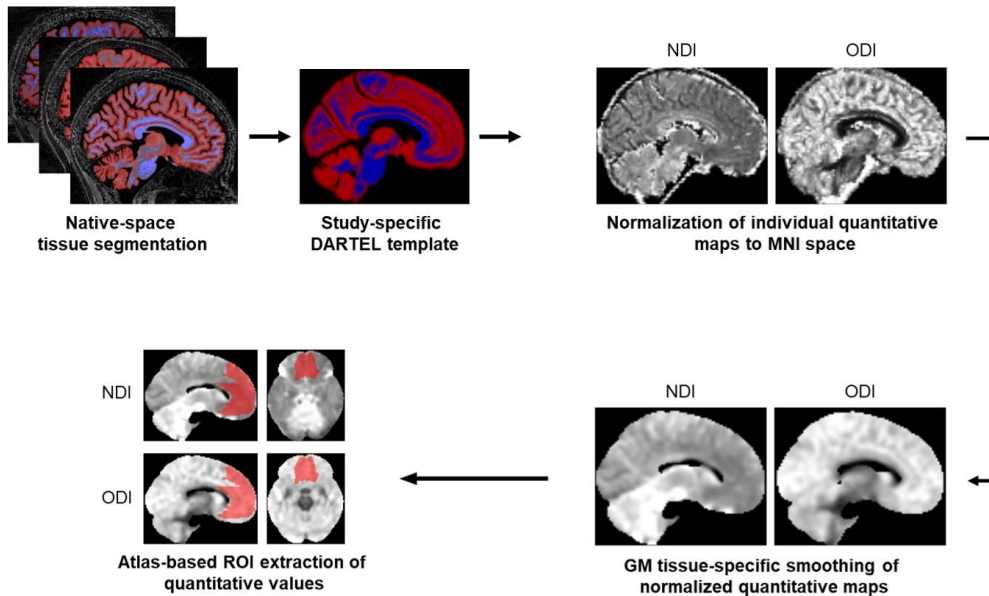
Supplementary table 1. Contingency matrix between the consensus of experts and automatic scoring on C4O2 for the whole dataset computed

Number of epochs (whole dataset)		Expert consensus (EXPERT_CON)						Total
		Artefact or disagreement	W	REM	N1	N2	N3	
Algorithm (ALGO)	Artefact	61	37	38	36	58	0	230
	W	604	1723	64	238	63	0	2692
	REM	664	38	2255	219	164	0	3340
	N1	225	56	109	183	100	1	674
	N2	1901	99	263	501	6086	270	9120
	N3	1187	6	3	0	808	1041	3045
	Tot	4642	1959	2732	1177	7279	1312	19101

Supplementary table 2. Sensitivity (Se), Specificity (Sp), Negative Predictive Value (NPV) and Positive Predictive Value (PPV) for agreement between experts and ALGO for each sleep stage on C4O2

		W	REM	N1	N2	N3
ALGO vs EXPERT_1	Se	83.0	81.6	12.3	82.4	63.4
	Sp	95.1	94.2	97.6	76.1	94.6
	PPV	69.6	72.5	41.5	71.3	72.5
	NPV	97.6	96.5	89.0	85.8	92.0
ALGO vs EXPERT_2	Se	81.8	81.2	14.0	75.0	79.3
	Sp	97.1	95.1	97.5	80.6	88.5
	PPV	81.7	77.3	37.9	81.1	34.4
	NPV	97.1	96.1	91.4	74.4	98.3
ALGO vs EXPERT_CON	Se	89.6	83.7	16.0	84.3	79.3
	Sp	97.0	96.4	98.0	84.0	93.7
	PPV	82.5	84.3	40.8	84.3	56.0
	NPV	98.4	96.2	93.1	83.9	97.8

Appendix 2. Supplemental data for chapter VII: Frontal grey matter microstructure is associated with sleep slow waves characteristics in late midlife



Supplementary figure S1. Schematic representation of the MRI processing pipeline. For each subject, structural MTsat map was segmented into tissue class maps using Unified Segmentation. The segmentation outputs were then diffeomorphically registered to a study-specific template created with DARTEL, and normalised to the Montreal Neurological Institute (MNI) space. Whole-brain NODDI parameter maps (NDI, ODI) were computed using the Microstructure Diffusion Toolbox, then registered to the MTsat map and brought into the MNI space by applying normalisation parameters estimated previously. Smoothing of normalised NODDI maps was performed using a tissue-specific weighting accounting for partial volume distribution of GM and WM tissue in each voxel, with a full-width at half maximum kernel of 6mm isotropic. For each individual, median regional NDI and ODI signals were obtained by applying a composite mask of the ROI (involving bilaterally, medial prefrontal, medial orbito-frontal, dorsolateral frontal, insula, and anterior cingulate cortices) based on the Automated Anatomical Labelling atlas (AAL2), applied on the normalised GM-weighted smoothed NODDI maps.

Appendix 3. Supplementary analysis for general discussion: Clustering analysis of arousals

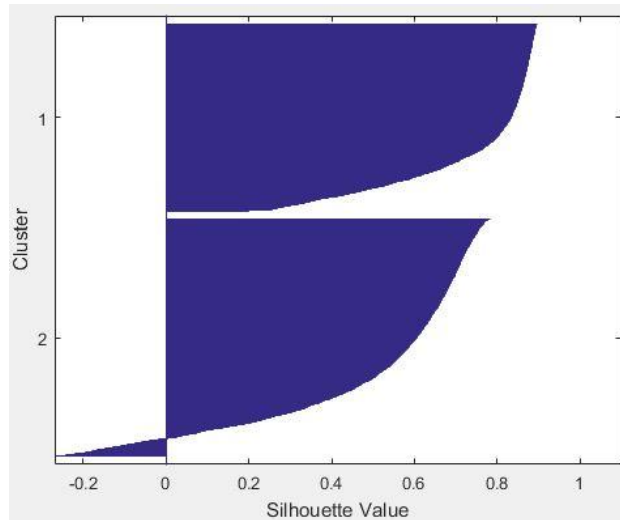


Figure 1. Silhouette plot of all arousal events over the whole dataset, proving the good quality of the two clusters

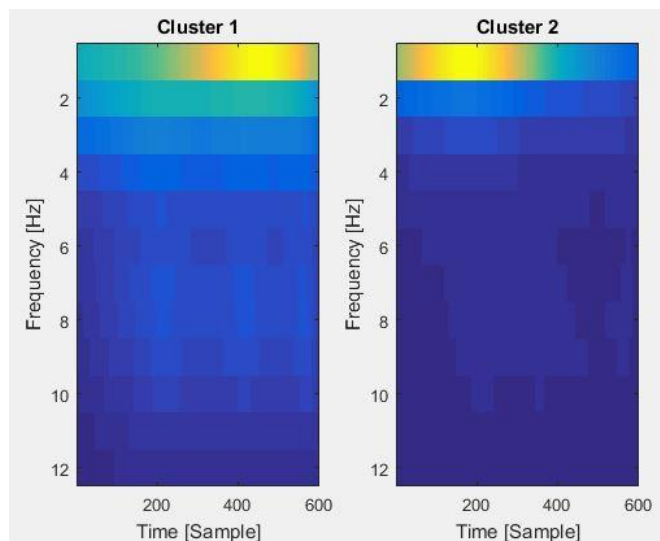


Figure 2. Time-frequency of arousals from cluster 1 and cluster 2, y axis shows frequency bins of 1Hz starting from 0.5 Hz to 12.5Hz, x axis shows time in samples, at a 200Hz sampling frequency (corresponding to 3 seconds).

Appendix 4. Supplementary analysis for general discussion: Clustering analysis of arousals

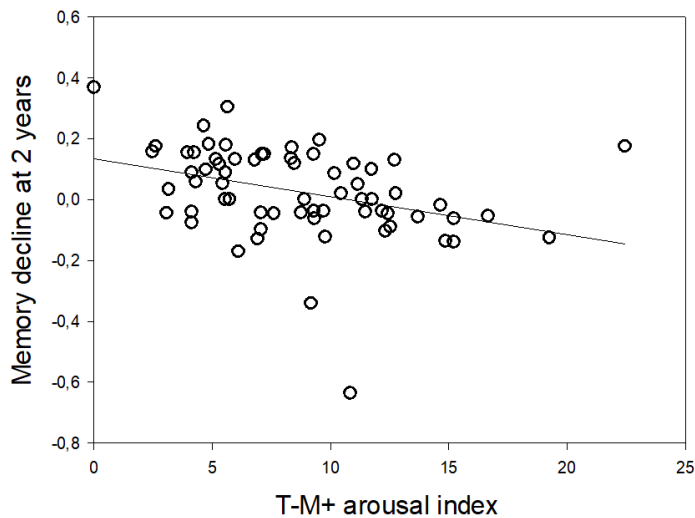


Figure 1. Negative association between the memory decline over 2 years as assessed by the MST and T-M+ arousal index

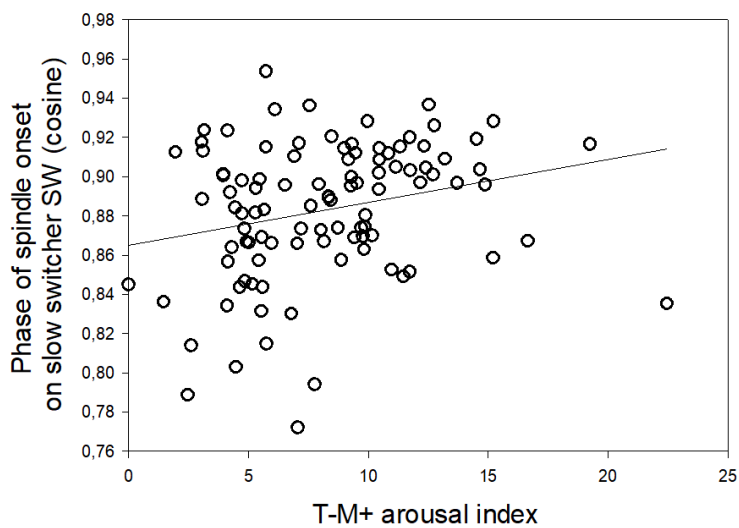


Figure 2. Positive association between the phase of spindle onset on slow switcher SW and T-M+ arousal index

